STUDIES ON THE EFFECTS OF SOME POLYPEPTIDES AND MONOAMINES ON THE CAROTID BODY CHEMORECEPTORS.

by

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Χαίρετε, λεπταὶ ῥήσιες, Ἀρήτου σύμβολον ἀγρυπνίης.

Kallimachos: ΕΠΙΓΡΑΜΜΑΤΑ.

Ἄρξομαι δὲ ἀπὸ τῶν προγόνων πρῶτον· δίκαιον γὰρ αὐτοῖς καὶ πρέπον δὲ ἅμα ἐν τῷ τοιῷδε τὴν τιμὴν ταύτην τῆς μνήμης δίδοσθαι.

Thoukidides: ΞΥΓΓΡΑΦΗ. II, 36.

DECLARATIONS.

Statement in terms of Ph.D. regulation 2.4.15 of the Postgraduate Regulations of the University of Edinburgh.

I declare that this thesis was totally composed by myself, and is based upon work carried out by myself, either alone or in collaboration with my supervisor, Dr D.S. McQueen. I contributed substantially to the design and performance of the experiments, and to the analysis and subsequent interpretation of results.

Some estimations of gas tensions and pH in arterial blood samples were performed by Mrs J.W. Gordon and Mrs R.A. Bond. Mrs Gordon also assisted in some of the analysis of computer-stored data, according to my instructions. The computer programmes used for data analysis were devised by Dr H.M. Brash (Department of Medicine, University of Edinburgh) and Mrs Bond (Department of Pharmacology, University of Edinburgh). Programmes used for statistical analysis of results were obtained commercially (R.B. Barlow (1984), Biodata Handling with Micro-computers, Elsevier-Biosoft, Cambridge).

October, 1985.

Statement in terms of Ph.D. regulation 2.4.11 of the Postgraduate Regulations of the University of Edinburgh.

The results of some experiments reported in this thesis have been published (see Appendix 3) or are awaiting publication, as follows:

Kirby, G.C. & McQueen, D.S. (1983). Changes in responses of cat carotid body chemoreceptors to 5-HT after administration of the antagonist MDL 72222. J. Physiol., 346, 96P.

Kirby, G.C. & McQueen, D.S. (1984). Effects of the antagonists MDL 72222 and ketanserin on the responses of cat carotid body chemoreceptors to 5-hydroxytryptamine. Br. J. Pharmac., 83, 259-269.

Kirby, G.C. & McQueen, D.S. (1984). Effects of a new 5-hydroxytryptamine (5-HT) antagonist, MDL 72222, on responses of cat carotid chemoreceptors to 5-HT and hypoxia. Proceedings of the 1984 Regional Meeting of the International Union of Physiological Sciences, Jerusalem, 26-31 August, 1984.

Kirby G.C. & McQueen, D.S. (1984). Effects of substance P on responses of cat carotid body chemoreceptors to dopamine, noradrenaline, and 5-hydroxytryptamine. Br. J. Pharmac. 83, 455P.

Kirby, G.C. & McQueen, D.S. (1985). Effects of selective β adrenergic antagonists on the response of cat carotid chemoreceptors to hypoxia. J. Physiol., 365, 102P.

Kirby, G.C. & McQueen, D.S. (1985). Effects of selective 5-hydroxy-tryptamine agonists on carotid body chemoreceptor discharge in anaesthetized cats. Br. J. Pharmac., In Press.

Kirby, G.C. & McQueen, D.S. (1985). ICI 154,129 antagonizes [Met]enkeph-alin-induced depression of cat chemosensory discharge. J. Physiol., In Press.

Kirby, G.C. & McQueen, D.S. (1985). Pharmacological studies on opioid receptors in the cat carotid body. To be published in the Proceedings of the VIIIth International Symposium on the Peripheral Arterial Chemo-receptors, Lisbon, 7-12 October, 1985.

ABSTRACT.

Carotid chemosensory activity was recorded from the peripheral end of the cut sinus nerve in anaesthetised cats and rabbits. The effects on chemoreceptor discharge of various putative transmitters were studied, and selective agonists and antagonists were used to characterise the pharmacological receptors involved in the responses observed.

Intracarotid injection of noradrenaline (NA) caused dose-related chemodepression via dopamine D_2-receptors; the effect was potentiated after administration of the β_1-adrenoceptor antagonists metoprolol or betaxolol. Chemodepression was followed in 67% of recordings by transient ('E_1') excitation, which was not blocked by potent adrenoceptor antagonists. When injected, NA also caused secondary ('E_2') excitation which correlated with an increase in blood pressure and heart rate. Excitation was also caused by the β-adrenoceptor agonists isoprenaline and salbutamol; it was concluded that this effect, and E_2-excitation evoked by NA, depended on changes in cardiac output, since both were reduced after selective antagonists which also blocked cardiovascular changes contributing to alterations in cardiac output evoked by adrenoceptor agonists. The α_2-agonist oxymetazoline caused excitation, but the α_1-agonist phenylephrine did not; both were equipotent with NA in causing hypertension. Vasoconstrictive hypertension alone does not, then, account for the 'vascularly'-mediated chemoexcitatory effect of NA. Infusion of NA depressed chemoreceptor firing, and was capable of suppressing the chemoreceptor response to hypoxia or hypercapnia; excitation was less commonly observed during

infusions of NA. The persistence of responses to hypoxia after adrenoceptor blockade implies that adrenergic mechanisms are not fundamental to chemoreception.

The use of novel 5-hydroxytryptamine (5-HT) antagonists showed that the occasional initial transient chemoexcitation, and the consistent depression of discharge evoked by 5-HT are mediated through receptors sensitive to the 'neuronal' antagonist MDL 72222, and the less intense, more delayed excitatory effect is mediated by receptors sensitive to the 5-HT_2-antagonist ketanserin. The response to hypoxia was largely unaltered after 5-HT antagonists, suggesting that 5-HT mediated mechanisms are not essential for chemoreception.

Opioid peptides evoked chemodepression, and the pharmacological profile was consistent with mediation of the effect through the 'δ' subtype of opioid receptor. Infusions of [Met]enkephalin depressed responses to hypoxia and hypercapnia. Potentiation of responses to hypoxia after the δ-selective antagonist ICI 174864 suggests that endogenous opioids may tonically inhibit chemoreceptor activity. Effects of [Met]enkephalin infusions on responses to injections of monoamines were equivocal, and better means of quantifying the effects observed are required before the possibility of interactions between monoamines and opioids can be determined.

The effects of substance P (SP) were indeterminate, but the potentiation of responses to 5-HT during SP infusion suggests further investigations of SP's possible role as a modulator of the actions of other transmitters should be attempted. An excitatory effect of

vasoactive intestinal polypeptide (VIP) was confirmed, but variable changes in the effects of monoamines injected during VIP infusions were observed.

Overall the results suggest that NA and 5-HT can affect the chemoreceptors both directly and indirectly, and, in common with polypeptides, are able to modulate chemosensory activity. Circumstances of their release in response to stimuli are awaited before final conclusions on their roles, if any, in arterial chemoreception can be reached.

LIST OF ABBREVIATIONS AND UNITS.

a.c.	alternating current
ACh	acetylcholine
ADR	adrenaline
ATP	adenosine triphosphate
ATR	atropine
β-END	β-endorphin
BET	betaxolol
BP	blood pressure (mm Hg)
b.p.m.	beats per minute
°C	degrees centigrade
CA	catecholamine
Ca^{2+}	calcium cation
cAMP	cyclic adenosine monophosphate
c.b.	carotid body
CHLORP	chlorpromazine
cm	centimetre
CN^-	cyanide anion
C.N.S.	central nervous system
CO_2	carbon dioxide
CONT	control
COR	corynanthine
c.p.s.	counts per second
d	delay (seconds)
D, Dep	chemodepression
DA	dopamine
DADL	enkephalin analogue (see Appendices 1 & 2)
DAGO	enkephalin analogue (see appendices 1 & 2)
DBZ	dibenzyline
DβH	dopamine-β-hydroxylase
d.c.	direct current
DCI	dichloroisoprenaline
DCV(s)	dense-cored vesicle(s)
DHE	dihydroergotamine
DIB	dibenamine
DOB	dobutamine
DP	domperidone
D-TC	D-tubocurarine
DYN	dynorphin octapeptide
Δ	'change in'*
ΔM, Δmax	'change' in maximum*
$\Delta\bar{x}$	'change' in the mean*; also 'mean difference'
$\Delta\bar{x}$%	'change in mean' expressed as percentage change
avΔ%	average percentage change•
$\bar{x}\Delta$%	percentage change in mean•
$\Delta\Sigma$ x	discharge integrated with respect to control*
E, Exc	chemoexcitation
E_1	early excitation (see Sect. 3)
E_2	delayed excitation (")
E.C.G.	electrocardiogram
ED_{30}	dose causing '30% excitation'
ELD	eledoisin
Em	membrane potential
ENK	enkephalin
EPSP(s)	excitatory post synaptic potential(s)
$ETCO_2$	end-tidal CO_2 (%)
ETZ	ethylketocyclazocine
Expt (No)	experiment (number)
F	Faraday's number
Fig(s)	Figure(s)
α-Flu	α-flupenthixol
g	gram
GTP	guancsine triphosphate
H^+,$[H^+]$	hydrogen ion (concentration)
$[^3H]$-	tritiated-
HAL	haloperidol
(N) HCl	(normal) hydrochloric acid
HCO_3^-	bicarbonate anion
hr	hour
HRP	horse radish peroxidase
5-HT	5-hydroxytryptamine
Hz	Hertz (unit of frequency)
$[^{125}I]$	radio-iodine label
i.a.	intra-arterial(ly)
i.c.	intracarotid(ally)
ID_{50}	dose causing '50% depression'
IPSP(s)	inhibitory post synaptic potential(s)
ICI...	see appendices 1 & 2
ISO	isoprenaline
i.v.	intravenous(ly)
K	kilo
K^+,$[K^+]$	potassium ion (concentration)
$[K^+]_i$	intracellular potassium ion concentration

* see p. 74; • see p. 299.

$[K^+]_o$	extracellular potassium ion concentration
L, Lk	Locke solution
[Leu]ENK	leucine enkephalin
ln	natural logarithm
log, $\log_{10}$	logarithm to base 10
LSD	lysergic acid diethylamide
m	metre
M	molar concentration
max	maximum
%max s^{-1}	% of maximum per second
MCPT	morphiceptin
MEC	mecamylamine
2Me 5HT	2-methyl 5-hydroxytryptamine
MET	metoprolol
[Met]ENK	methionine enkephalin
MDL	MDL 72222 (App. 1 & 2)
mg	milligram
Mg^{2+}	magnesium cation
µg	microgram
min(s)	minute(s)
ml	millilitre
µl	microlitre
mm, mm^3	millimetre (cubed)
mm Hg	millimetre of mercury
µm	micrometre
mM, mmol	milli-mole/-molar
mol	mole
5-MOT	5-methoxytryptamine
µM, µmol	micro-mole/-molar
ms	millisecond
mV	millivolt
n	number of observations
N_2	nitrogen
Na^+	sodium cation
NA	noradrenaline
NaCN	sodium cyanide
NAL	naloxone
ng	nanogram
nm	nanometre
nmol	nano-mole/-molar
O_2	oxygen
6-OHDA	6-hydroxydopamine
8-OH DPAT	- see appendices 1 & 2
OXM	oxymetazoline
P	statistical probability
PB	pentobarbitone
PCO_2 $PaCO_2$	(arterial) CO_2 tension (mm Hg)
PERPH	perphenazine
pg	picogram
PHENB	phenoxybenzamine
PHEN	phenylephrine
PHENT	phentolamine
PHY	physalamin
pmol	picomole
PO_2, PaO_2	(arterial) O_2 tension (mm Hg)
PRAZ	prazosin
PREN	prenalterol
PROP	propranalol
R	the gas constant
r	correlation coefficient
RAU	rauwolscine
RU24969	see appendices 1 & 2
s	second
SAL	salbutamol
s.e.m.	standard error of the mean
SK	substance K (neurokinin α)
SP	substance P
SPIR	spiroperidol
SP-O-Me	methyl ester of substance P
Σx	total counts*
t	'duration' (seconds)*
T	absolute temperature
TH	tyrosine hydroxylase
VIP	vasoactive intestinal polypeptide
w/v	weight per volume
$\bar{x}$	arithmetical mean
~	approximately

* see p. 74

Most abbreviations, other than commonly used expressions, are also defined at the first point of occurence in the text; the above list may be used for easy reference.

CONTENTS

SECTION 1

GENERAL INTRODUCTION

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GENERAL INTRODUCTION.

The mammalian carotid body is a relatively small sensory organ (about $1mm^3$ in the cat) located bilaterally at the bifurcation of the common carotid artery (see Fig. 1.1). Its location, gross structure, and innervation is remarkably similar between species (cf. Adams, 1958), although considerable variations can occur from one individual to another.

Situated within the carotid body are arterial chemoreceptors which can be activated by low PaO_2, high $PaCO_2$ or decreased arterial blood pH; their activity is also altered by changes in temperature and tonicity (the physiological significance of which is uncertain) and by the exogenous administration of substances such as cyanide (CN^-), acetylcholine (ACh), or dopamine (DA); these latter two substances have established roles as neurotransmitters in various parts of the nervous system, and are also present in the carotid body. The carotid body chemoreceptors are important in homeostasis, mediating reflex effects upon the CNS through sensory discharges of the carotid sinus nerve.

The functional elements of the carotid body are the 'type I' cells, 'type II' cells, and sensory nerve endings, but there is little agreement as to the identity of the primary receptor element, or the mechanisms responsible for the induction of sensory discharge in the sinus nerve. Stimuli acting upon the type I cells may release the 'transmitter' substances that they contain, but it is unknown whether released chemicals are required for the initiation of sensory

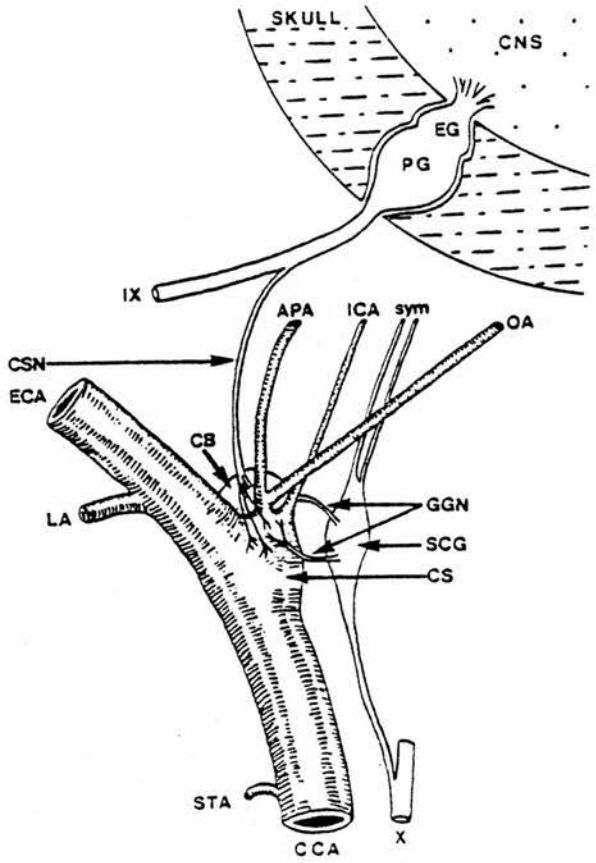

Figure 1.1: Schematic representation of the left carotid bifurcation of the cat, and the nerves associated with the carotid body (adapted and re-drawn from Adams, 1958).

APA: ascending pharyngeal artery;
CB: carotid body;
CCA: common carotid artery;
CNS: central nervous system (i.e the brain stem);
CS: carotid sinus;
CSN: carotid sinus nerve;
ECA: external carotid artery;
GGA: ganglioglomerular nerves;
ICA: internal carptid artery;
IX: glossopharyngeal nerve;
LA: lingual artery;
OA: occipital artery;
SCG: superior cervical ganglion;
STA: superior thyroid artery;
sym: cervical sympathetic trunks;
X: vagus nerve;
PG: petrosal (Andersh's) ganglion, of which the rostral part (EG) is also known as Ehrenritter's (or the superior) ganglion;

The glossopharyngeal nerve is usually exposed when the cannulated lingual artery is deflected towards the superior cervical ganglion; the carotid sinus nerve is then dissected out from beneath the common carotid artery, and the recording electrodes positioned between the artery and the mid-line.

impulses, or merely serve to modulate an already ongoing discharge, which could be a property of spontaneously active nerves. This study is concerned specifically with the effects of neuroactive polypeptides and monoamines upon carotid body chemoreceptor activity in the cat, with the inclusion of the results of a small number of experiments carried out in rabbits. The purpose of this work was to elucidate the actions of these substances (which have been shown to be present in the carotid body), their interactions with one another and their effects upon the response of chemoreceptors to 'natural' stimuli such as hypoxia. Experiments were designed to provide information as to whether these substances could act as chemical transmitters in the basic processes of chemosensory transduction. The remainder of this introduction describes some of the previous studies on the structure and function of the carotid body, together with the main theories of chemotransduction that have been proposed.

1.1 Early studies on structure and function.

Luschka (1862) accorded the first anatomical description of a carotid body to von Haller (1762), although there are earlier descriptions of a structure at the carotid bifurcation (e.g Taube, 1743). Pförtner (1865) appears to be the first to have described the carotid body in the cat. Luschka's own opinion was that the carotid body is a gland, whereas Arnold (1865) considered it to be merely a vascular organ. The demonstration of chromaffin cells by Kohn (1900) led him to define the carotid body as a paraganglion, an idea which might be suggested both by the close proximity of the carotid body to the superior cervical and the nodose ganglia, and by the clear sympathetic innervation of the carotid body and the adjacent sinus region

by fibres from the superior cervical ganglion, the ganglioglomerular nerves.

The nature and function of the carotid body were at times greatly disputed or relegated to obscurity until De Castro, in the late 1920's, laid the foundations for the current interest in and studies of the carotid body structure and function. He demonstrated histologically the innervation of the carotid body by a branch of the glossopharyngeal nerve, and the presence of nerve endings on the cells of the carotid body parenchyma (1925, 1926). His studies revealed a spatial arrangement of 'type I' cells within the carotid body, one pole being adjacent to a blood vessel ('pole sanguine'), the other being associated with nerve terminals ('pole nerveux'). By 1928 he had clearly differentiated baroreceptors in the 'sinus caroticum' wall from the chemosensory receptors in the carotid body itself, and had shown experimentally that the innervation of the carotid body is afferent, or sensory (rather than efferent, or motor), leading to his prediction that "the 'glomus caroticum' represents a sense organ that detects qualitative variations in the blood, a function which, by reflex action, will have an influence on the functional activity of other organs".

His interpretation of the carotid body as a chemosensory organ was confirmed physiologically by Heymans et al (1930) and by Heymans & Bouckaert (1930) who showed the carotid body to be the site of an arterial chemoreceptor system sensitive to the chemical composition of the blood, which, when stimulated, initiates respiratory and cardiovascular reflexes (see Table 1.1). This function had been noted, but not understood, by Pagano (1900) who observed reflex hyperventilation and bradycardia when either prussic acid or nicotine was injected into

Table 1.1: Reflex effects resulting from stimulation of carotid body chemoreceptors.

A. Respiratory effects	
1. Increased tidal volume, frequency, and minute volume	Heymans et al, 1930 and Heymans & Bouckaert, 1930
2. Increased bronchiolar tone	Daly & Schweitzer, 1951
B. Cardiovascular effects	
1. Bradycardia	Daly & Scott, 1958
2. Increased pulmonary vascular resistance	Daly & Daly, 1959
3. Systemic vasoconstriction	Daly & Ungar, 1966
C. Other effects	
1. Increased motor cortex activity	Schmidt & Comroe, 1940
2. Increased secretion from adrenal medulla and cortex	Anichkov et al, 1960

the common carotid artery in dogs. No such reflex was obtained if the injection was made into the internal carotid artery, bypassing the carotid body.

1.2 Peripheral arterial chemoreceptors.

It is generally accepted that the systemic effects of both hypercapnia and acidaemia are exerted mainly through actions upon respiratory 'centres' in the medulla and brain stem, whereas the systemic response to hypoxia is dependent, almost exclusively, upon the integrity of the peripheral arterial chemoreceptors. Carotid chemoreceptor activity accounts for some 40% of the respiratory response to hypercapnia during hyperoxia (Berkenbosch et al, 1979). Even so, hypercapnia or acidaemia, in the presence of hypoxia, exert a more than additive effect upon chemoreceptor discharge (Otey & Bernthal, 1960; Eyzaguirre & Lewin, 1961; Joels & Neil, 1961), and the elimination of central chemosensitivity (by coagulation of ventral medullary areas driving respiration in response to H^+ ions - Schlaefke & Loeschke, 1967) results in total dependence of respiratory activity upon peripheral chemoreceptor function (Schlaefke et al 1969). It has been proposed (Schlaefke et al, 1979) that central chemosensory function amplifies the responses to hypoxia and hypercapnia mediated by the peripheral chemoreceptors.

Arterial chemoreceptor function is not confined to the carotid body, but is also found in the aortic arch region ('aortic bodies' - Diamond & Howe, 1956; Paintal & Riley, 1966; Abbott & Howe, 1972; Sampson & Hainsworth, 1972), and in 'miniglomera' found in the region of the carotid bifurcation, though separate from the carotid body itself (de Castro, 1962), and, in the cat at least, around the common

carotid artery (Matsuura, 1973). It appears that both physiological and ultrastructural features of such miniglomera are identical to those of carotid and aortic bodies, and they are innervated, like the aortic bodies, by fibres projecting from the nodose ganglion. Abdominal chemoreceptors have also been identified (e.g Hollinshead, 1941), but only in the mouse and rat, which have poorly developed, or no aortic bodies. These structures are histologically identical with the carotid body (Andrews et al, 1972) and have been shown to mediate a consistent but slight chemoreflex in mice (Hollinshead, 1946); this chemoreflex is variable in the rat and absent in the cat, guinea-pig and rabbit (Hollinshead, 1946). Chemosensory activity has been recorded from filaments of the vagus nerve innervating these 'vagal paraganglia' in the rat (Andrews et al, 1972; Howe et al, 1981).

1.3 Elements constituting the chemosensory complex.

The term 'glomoid' (Seidl, 1975) appears to be gaining favour for describing the functional arrangements of capillaries, cells, and nerve endings. The non-neural components of the glomoid are clusters of cells associated with the capillaries (see Fig. 1.2). Groups of cells in intimate apposition to the capillaries are invested with collagenous connective tissue, through which many myelinated and non-myelinated nerve fibres are seen to pass; nerve endings impinge upon both cells and blood vessels.

1.3.1 Vascular elements.

1.3.1.1. Blood supply.

The blood supply to the carotid body is from a small arterial

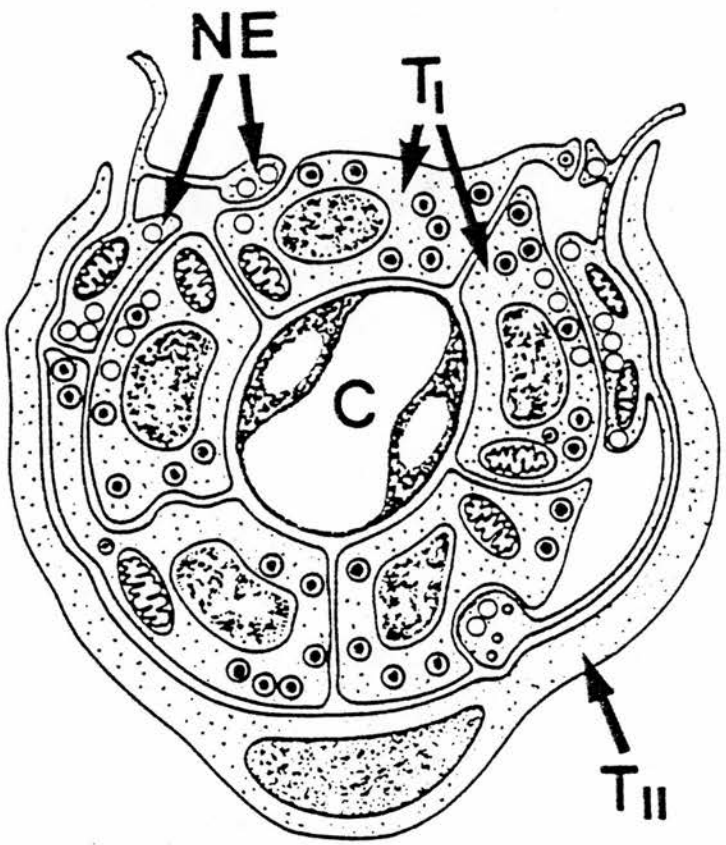

Figure 1.2: Schematic representation of a 'glomoid' (Seidl, 1975), taken from Eyzaguirre and Zapata (1984).

Type I cells (TI) surrounding a fenestrated capillary (C) are innervated by sensory nerve endings (NE), which are the terminals of fine branches of the carotid sinus nerve. Nerve terminals contain clear-cored vesicles (O), whilst type I cells are characterised by dense-cored vesicles (⊙), surrounded by a clear 'halo'; type I cells also contain clear-cored vesicles, particularly in the vicinity of nerve terminals. This arrangement of type I cells and nerve endings is almost completely enveloped by processes of type II (TII) cells.

branch originating from some aspect of the bifurcation (see Adams, 1958; Seidl 1976), which attaches the carotid body to the artery, and may project through the carotid body to supply adjacent structures, particularly the superior cervical ganglion (Chungcharoen et al, 1952). The venous drainage originates in a capsular plexus, from which three or four veins emerge, to run into larger veins in the vicinity. Both arteries and veins may receive a barosensory innervation (de Castro, 1940; Ábrahám, 1968).

1.3.1.2. Innervation of blood vessels.

Carotid body blood vessels are extensively innervated both by sympathetic nerves, with their cell bodies in the superior cervical ganglion, and by parasympathetic fibres, the cell bodies of which are found in the carotid body (McDonald & Mitchell, 1975a,b). Besides conventional transmitters, there is evidence that vasoactive intestinal polypeptide (VIP) and substance P (SP) are found in the terminals of nerves making contact with blood vessels (Lundberg et al, 1979a; Wharton et al, 1980; Cuello & McQueen, 1980).

1.3.1.3. Arteriovenous anastamoses.

Tissue oxygen levels can vary in different parts of the carotid body (Acker & Lübbers, 1975; Whalen & Nair, 1975) and local flow in the organ can be altered independently of total flow (e.g. Acker & Lübbers, 1975). De Castro and Rubio (1968) observed that hypoxia, anoxia, and hypercapnia decreased blood flow through the carotid body and reduced its size; conversely, breathing a hyperoxic gas mixture increased blood flow through the capillary network, and enlarged the organ. These changes appear to result from the opening and closing of

the arteriovenous anastamoses which are a prominent feature of the carotid body structure (Goormaghtigh & Pannier, 1939; de Castro, 1940) and which arise where some of the small terminal arterioles feed directly into small venules (Serafini-Fracassini & Volpin, 1966; Schäfer et al 1973).

Using more refined methods, Acker (1980) demonstrated a decrease in local blood flow during hypoxia, but not in response to hypercapnia. Autonomic stimulation had no effect upon local blood flow or PO_2 within the carotid body, although total blood flow through the organ was decreased by the vasoconstriction elicited by stimulation of the sympathetics, and increased vasodilatation resulted from stimulation of the peripheral end of the cut sinus nerve (Acker & O'Regan, 1981). The autonomic nerves appear to control the arteriovenous anastamoses which thus act as 'shunts', whilst other mechanisms must regulate the local flow within the carotid body capillary network, independently of total blood flow, by an as yet ill-defined PO_2-dependent mechanism (Acker, 1980).

1.3.1.4 Capillaries.

The 'functional' vasculature of the carotid body is to be found in the capillary network (e.g. Seidl, 1976). These capillaries, which have a continuous fenestrated epithelium (and are thus not simply intercellular sinusoids), are in intimate contact with the 'type I' cells (Fig. 1.2). Variability in the diameter of the lumen has been noted (Seidl, 1975) so that considerable changes in blood flow velocity might occur at different points of the network.

1.3.2 Cellular elements.

1.3.2.1. Type I cells.

Most numerous of the cells, which fall broadly into two types (cf. Fig. 1.2), are those of 'type I' (also termed 'glomus', 'chief', 'epithelioid', or 'granular'). These cells are small (~10 μm), with a large nucleus containing diffuse, lightly-staining chromatin. They are generally enveloped by the 'type II' cells ('interstitial', 'sustentacular', 'enclosing', or 'satellite' cells), and extend cytoplasmic processes towards other cell clusters or towards capillaries (Nishi, 1976). They exhibit many of the features of secretory cells: abundant mitochondria, much endoplasmic reticulum, Golgi bodies, and single or polysomic ribosomes, and are characterised, in particular, by the presence of many large (30-120 nm) dense-cored vesicles (DCVs) that are similar to, but smaller than, those present in the adrenal medulla (Hellström, 1975a; Hess, 1968; Lever & Boyd, 1957; Lever et al 1959; Verna, 1977, 1979). It is generally accepted that these vesicles are the site of storage of catechol- and indole- amines (see later), since labelled catecholamine precursors are taken up by the vesicles (Chen & Yates, 1969) as are 'false' precursors such as 5-hydroxydopa and 6-hydroxydopamine (Hellström, 1975b; Hess, 1976). They appear also to shrink after catecholamine depletion by reserpine (Hess, 1977b).

These vesicles also contain Ca^{2+}, ATP, and proteins (Chen et al, 1969), and it has been suggested that the carotid body might exercise a secretory function (Karnauchow, 1965; Pearse, 1969; Capella & Solcia, 1971; Kobayashi, 1975), either related to, or independent of the chemoreceptor function. However, autoradiographic studies using tritiated leucine, monoamines, and ATP have shown that the turnover of

these substances in type I cells is slow in comparison with the turnover of secretory products in endocrine cells of the adrenal medulla or gut (Kobayashi, 1976, 1977).

Type I cells in a glomoid are separated one from another, and from the type II cells by an intercellular space of about 20 nm, which is accessible to vascularly injected horseradish peroxidase (HRP; Woods, 1975). Junctions have been observed between adjacent type I cells in several species (e.g. Biscoe & Stehbens, 1966; Hess, 1968, 1975a), but especially in the rat (Hess, 1975a; McDonald & Mitchell, 1975a; Morgan et al, 1975). Also, in the rat, more specialised junctions have been described, and it has been suggested from their morphology, and the presence of apparent synaptic vesicles that these are 'synapses' (McDonald & Mitchell, 1975a). The term 'synapse' is only properly applied to a junction between two nerves, but its use is perhaps justified in this instance if the type I cells are considered to be 'paraneurones' - see below. A similar junction has been described between a type I and a type II cell (Hess, 1975a), but the accumulation of vesicles reported at the membrane of type II cells abutting the interstitial connective spaces (Verna, 1979) is hardly suggestive of a synapse. More well defined are the junctions between type I cells and the sensory nerve endings (see later).

The histochemical demonstration of neurone-specific enolase in type I cells (Kondo et al 1982), indicates that these cells are derived from neural tissues and may thus be classified as 'paraneurones' (Fujita & Kobayashi, 1979). The paraneurone concept envisages a 'continuity' of neurones and endocrine or secretory cells, which are considered to form a continuous spectrum with regard to structure (presence of neurosecretion-like vesicles), products

(peptides, amines, neurohormones, and neurosecretions), and function (recepto-secretory).

The type I cells can also be classified as APUD cells (Amine Precursor Uptake and Decarboxylation cells - Pearse, 1969), which are derived from neuroendocrine-programmed cells originating from the ectoblast. Such cells characteristically produce peptides with hormonal or neurotransmitter activity, as well as bio-active amines. They could constitute a third division of the nervous system, supporting, modulating, or amplifying the actions of neurones in the somatic and autonomic divisions. According to Pearse (1978) the type I cells of the carotid body could be considered as 'neurocrine' in function - cells secreting into or onto neurones.

Both concepts have been formulated to smooth out some of the anomalies that surround non-neuronal cells which appear to have some important role in nerve function.

1.3.2.2 Type II cells.

Type II cells are some 3-5 times less numerous than type I cells (Biscoe & Pallot, 1972; McDonald & Mitchell, 1975b), which they surround with a thin cytoplasmic layer, sometimes only 0.2 μm thick (Verna, 1979). Cytoplasmic extensions have been seen to intrude between adjacent type I cells, and to project towards the centre of the cluster; this appears to occur more commonly in the cat than in the rat carotid body.

The flattened, often lobulated, nucleus is more electron-dense than that of type I cells, the chromatin being more compact. The cytoplasm contains much the same organelles as the type I cell, but DCVs are characteristically absent, as is the capacity to take up and

store tritiated monoamines.

The envelopment of nerve endings and type I cells by type II cells is analagous to the investment of nerve cells and neurones by glial or Schwann cells. A certain proportion of the membrane of both nerve endings and type I cells is not covered by type II cell processes, but remains exposed to surrounding connective spaces through a basement membrane. In man there is evidence that the number of type II cells can be increased by prolonged hypoxaemia or systemic hypertension (Heath et al, 1982).

1.3.3 Neural elements.

1.3.3.1 Afferent (sensory) innervation.

De Castro (1926), Biscoe & Stehbens (1967), Hess (1968), and Hess & Zapata (1972) showed that transection of the glossopharyngeal nerve leads to degenerative changes in nerve fibres with terminals associated with type I cells. Some junctions between nerve terminals and type I cells persist after this procedure (Hess & Zapata, 1972), and the nerve terminals are most likely sympathetic preganglionic fibres from the ganglioglomerular nerves (McDonald & Mitchell, 1975a,b).

Electron microscopy studies (de Castro & Rubio, 1968) showed that fibres innervating type I cells have their cell bodies in the sensory (petrosal) ganglia (see Fig 1.1). Axoplasmic flow studies may be used to trace neuronal projections (Lasek et al, 1968), and tritiated amino acids administered to the petrosal ganglion (Fidone et al 1975; Fidone et al 1976; Smith & Mills, 1976) were detected in the nerve endings making contact with type I cells, confirming that they originate from cells in the petrosal ganglion. Antidromic application of

HRP to the carotid sinus nerve (Berger, 1980) showed 96% of the cell bodies to be located in the caudal part of the petrosal ganglion, and the remaining 4% in the rostral part. The use of antidromic mapping techniques (Jordan & Spyer, 1977) has shown that the chemoreceptor afferent activity projects mainly to the ventrolateral part of the nucleus tractus solitarius; this area of the medulla also contains the 'dorsal' group of inspiratory neurones (Merrill, 1974). The 'lateral' group of inspiratory neurones appear not to receive a direct input from the carotid chemoreceptors (see Mitchell & Herbert, 1974). It remains to be established whether it is the frequency or the pattern of impulses that is important for the coding of chemosensory information at the level of the respiratory and cardiovascular centres in the brain stem.

The carotid sinus nerve is composed of myelinated (mainly A type) and non-myelinated C type fibres. Both chemoreceptor and baroreceptor afferents are found in this nerve, each modality being transmitted by both A and C fibres. Fidone and Sato (1969) estimated that about 61% of the A fibres in the cat sinus nerve are chemoreceptor afferents and 39% are baroreceptor afferents, whilst of the C fibres, some 17% of the total population appear to be chemoreceptor afferents, 29% baroreceptor afferents, and the remaining 54% are efferent in origin, both sympathetic and non-sympathetic. The ratio of myelinated to non-myelinated fibres appears to vary along the length of the sinus nerve (Eyzaguirre & Uchizono, 1961), and it has been suggested (Laurent & Jäger-Barrès, 1964) that unmyelinated fibres are the terminal parts of myelinated fibres. Generally, the threshold of A fibres is lower than that of C fibres, the response latency shorter, the acceleration of discharge more rapid, and the discharge frequency higher, regardless

of whether their modality is chemoreceptor or baroreceptor.

Chemoreceptor fibres (whether A or C type) show a characteristically irregular discharge under normoxic conditions in anaesthetised animals. This 'aperiodic' discharge (Eyzaguirre & Lewin, 1961) is characterised by the random distribution of the interval between impulses. Intervals between action potentials have been measured in normally circulated and in perfused carotid bodies (Biscoe & Taylor, 1963; Biscoe, 1965), and were found to be distributed exponentially, with the standard deviation of the intervals equal to the mean, thus satisfying two mathematical criteria for randomness. The pattern of discharge of A fibres tends to become more regular as frequency increases above 10 impulses s^{-1} (Biscoe & Taylor, 1963). Some A fibres may exhibit a regular pattern of discharge even at low rates of firing (Fidone & Sato, 1969); the conduction velocity of these particular fibres was observed to be at the lower limit of their calculated range for A fibres, 4-53 m s^{-1}.

1.3.3.2 Synaptic contacts.

Nerve endings on type I cells are morphologically complex (Nishi & Stensaas, 1974), and no consensus exists as to the type of terminals present (e.g. calyciform endings, boutons, 'en-passant' arrangements, cf. Verna, 1979). It is likely (Biscoe & Pallot, 1972; Nishi & Stensaas, 1974; Eyzaguirre & Gallego, 1975; Kondo, 1976) that any one afferent fibre will innervate several type I cells, after branching, possibly by means of terminals of differing size and form.

Many synaptic-like microvesicles are present in the nerve terminals (Nishi & Stensaas, 1974; McDonald & Mitchell, 1975[a,b], 1976; McDonald, 1977), the abundance of which is reduced by stimulation of

chemoafferent activity by hypoxia. It is unknown whether these vesicles contain a synaptic transmitter.

Various kinds of junction between nerve endings and type I cells have been described: i) 'efferent-like' junctions, with an accumulation of vesicles on the neural side (e.g. Biscoe & Stehbens, 1966; Kobayashi, 1971; Smith & Mills, 1976); ii) 'afferent-like' junctions, in which the accumulation of vesicles is on the type I cell side (Verna, 1979), and iii) the so-called 'reciprocal synapses' (McDonald & Mitchell, 1975a,b; Smith & Mills, 1976), which are not particularly numerous, and are better described as 'paired' junctions of opposite polarity, since they appear to represent the occurence of an 'efferent' junction close to an 'afferent' junction. Structurally, junctions between type I cells and nerve endings are similar to chemical synapses in the nervous system (Verna, 1979).

1.3.3.3 Denervation studies.

Sectioning of the sinus nerve appears to produce little alteration in the ultrastructure of type I cells (Biscoe & Stehbens, 1967; Abbott et al 1972; Hess, 1977a), but catecholamine stores become less sensitive to depletion by reserpine (Hess 1975b), from which it might be deduced that the intact sinus nerve exerts a retrograde or trophic effect upon type I cells. Centrifugal activity in the sinus nerve also appears to influence DCV content of type I cells (Yates et al, 1970), and the synthesis and release of catecholamines (Mills & Slotkin, 1975; Sampson et al, 1975). Type II cells appear to undergo hypertrophic and proliferative cytoplasmic changes after denervation of the carotid body (Hess, 1977a), but the significance of the effect is uncertain.

1.3.3.4. Efferent (non-sensory) innervation.

In addition to the afferent innervation of type I cells, they may be innervated from several other sources. Ganglion cells intrinsic to the carotid body, although few in number (about 1 per 348 type I cells in the rat - McDonald & Mitchell, 1975b) may establish contact between their dendrites and type I cell processes (Kondo, 1976).

In the rat perhaps 4% of the nerve endings on type I cells appear to originate from preganglionic, possibly cholinergic fibres (McDonald & Mitchell, 1975b). In the cat, however, extirpation of both the nodose and the superior cervical ganglia has no significant effect upon the number of nerve endings on type I cells (McDonald & Mitchell, 1981).

Postganglionic sympathetic fibres from the superior cervical ganglion innervate not only blood vessels (Biscoe & Stehbens, 1966, 1967; Rees, 1967; McDonald & Mitchell, 1975b), but also type I and type II cells (Kondo, 1971; Verna, 1975). These fibres may project to the carotid body via the ganglioglomerular nerves, or as postganglionic fibres leaving the superior cervical ganglion and travelling to the carotid body in the sinus nerve; they may also arise from sympathetic ganglion cells located in the carotid body itself (Verna, 1979).

Stimulation of the sympathetic supply may cause inhibitory, or excitatory effects upon afferent discharge (O'Regan, 1977). Excitation is either slow in onset, susceptible to α-adrenoceptor blockade, and thus attributable to a vasoconstrictor effect, or it is transient, resistant to blockade with α-adrenoceptor antagonists, and so non-vasomotor in nature. The possibility exists that noradrenaline (NA) released from these nerves may reach the chemosensory mechanism

resulting in stimulation.

De Castro (1926) provided histological evidence of efferent fibres in the carotid sinus nerve, and action potentials have been recorded from the central end of the cut sinus nerve by Biscoe and Sampson (1967, 1968), who showed two types of efferent activity: one group of neurones, with cell bodies in the superior cervical ganglion, displayed a cardiac or respiratory rythmicity in their pattern of discharge, whilst a second group of fibres showed a non-rythmical pattern of activity, but increased their firing in response to systemic hypoxia, hypercapnia, or intravenously injected adrenaline (ADR). Others have confirmed these findings in cats (Neil & O'Regan, 1969, 1971b; Majcherczyk et al, 1974; Willshaw, 1975) and in the rabbit (Laurent & Jäger-Barrès, 1969), and by recording from the central end of the cut aortic nerve (Neil & O'Regan, 1971b) which innervates the aortic chemoreceptors.

The cell bodies of these neurones have not been succesfully located using the methods already described for tracing neuronal projections, but would appear to be sited in the brain stem (cf. de Groat et al, 1979; Berger, 1980). Electrical stimulation or hypoxic activation of this efferent pathway causes depression of afferent activity (Sampson & Biscoe, 1970; Neil & O'Regan, 1971a,b). Goodman (1973) attributed this effect to antidromic depression or to vasomotor effects. Although vascular effects may be partially responsible for 'efferent inhibition', such inhibition has been demonstrated in the ischaemic carotid body (O'Regan, 1975), where there is no blood flow, and hence, no vasomotor activity, and, according to Willshaw (1975), efferent inhibition is not accompanied by significant changes in blood flow. McDonald and Mitchell (1981) showed the antagonism of efferent

inhibition by haloperidol and dihydroergotamine, and suggested the effect was due to antidromic stimulation of the sensory nerves, resulting in release of dopamine (DA). Belmonte and Eyzaguirre (1974) and McCloskey (1975) did not observe efferent inhibition in the absence of carotid body perfusion, but the possibility exists that there are efferent inhibitory mechanisms which are not mediated by alterations in blood flow.

1.4 Theories of the mechanism of chemotransduction.

Pharmacological studies using selective neurotranmitter agonists and antagonists have proved valuable in establishing the nature of chemical transmission occuring in the motor nervous system, and these techniques have been widely adopted in investigating the mechanisms involved in the sensory process of chemoreception in the carotid body, where the release of transmitter (generator) substances has been postulated (e.g. de Castro, 1928; Eyzaguirre et al, 1972). The case for chemical transmission in the carotid body has yet to be proved, and hypotheses exist (see later) which, whilst attempting to explain chemoreception without invoking chemical transmission, may also be considered as mechanisms underlying the release of transmitter substances.

1.4.1. Information provided by the nature of the hypoxic stimulus.

The prime stimulus of an increase in the frequency of chemoreceptor discharge is hypoxia. Four differing types of hypoxia have been defined: i) hypoxic - low PaO_2; ii) anaemic - normal PaO_2, but with low haemoglobin content; iii) stagnant - normal PaO_2, but with low blood flow, low O_2 flow, and tissue 'asphyxia'; iv) histotoxic -

normal PaO_2, but with poisoning of the cytochrome enzyme system of cellular oxidation.

Hypoxic or histotoxic hypoxia of the perfused carotid body provokes chemoreceptor reflexes (Heymans & Rijlant, 1933). In contrast, anaemic hypoxia, as a result of formation of carboxyhaemoglobin (Comroe & Schmidt, 1938) does not cause reflex hyperpnoea or hypertension, even when 70% of the haemoglobin content of the blood has been converted to carboxyhaemoglobin (Duke et al, 1952). Comroe and Schmidt (1938) suggested that at normal PaO_2 (~100 mm Hg) the small amount of O_2 carried in physical solution in the plasma (3 ml O_2 per litre of blood) is sufficient to satisfy the oxygen usage of the carotid body. Direct measurements of carotid and aortic chemoreceptor activity in the cat (Lahiri et al, 1981b) have shown that most carotid chemoreceptors are not stimulated by 60% carboxyhaemoglobinaemia at 'normal' PaO_2 (82-98 mm Hg) and isocapnia ($PaCO_2$ ~30 mm Hg). The aortic chemoreceptors are, however, stimulated by even 10% carboxyhaemoglobinaemia, and the response is augmented by lowering PaO_2.

Chemosensory discharge in the carotid sinus and aortic nerves increases in response to stagnant hypoxia resulting from haemorrhagic hypotension (Landgren & Neil, 1951). Neil (1951) proposed that the type I cells are ordinarily maintained on the verge of anaerobic metabolism, but that blood flow is normally sufficient for the dissipation of released anaerobic metabolites. Under conditions of reduced blood flow the accumulation of these metabolites would cause excitation of the chemosensory mechanism. From this idea stems the general concept that chemosensory discharge originates from the supply or removal of neuroactive substances.

1.4.2 Oxidative phosphorylation as a determinant of chemoreception.

If the carotid body is perfused with hypoxic-hypercapnic, or cyanide-containing solutions, the elevation of discharge gradually declines to a less active steady-state in which the application of ACh (which stimulates sensory nerve endings directly) provokes a brisk chemoreceptor stimulation, proving that the nerve endings remain capable of initiating action potentials (Joels & Neil, 1962). It may be inferred that in this state the type I cells have been exhausted of something that mediates the elevated discharge in response to hypoxia; perfusion with ATP-containing solutions will offset this exhaustive process (Joels & Neil, 1963), and both Anichkov (1951) and Belen'kii (1951) have argued that chemosensory activity is evoked by reduced concentrations of cellular ATP and other high-energy phosphate cont-ning compounds. The uncoupling of oxidative mechanisms from ATP synthesis (e.g. using 2,4-dinitrophenol) causes marked chemoreceptor stimulation (Joels & Neil, 1963).

1.4.3. Possible function of type I cells as the chemotransducer.

De Castro (1928) and Eyzaguirre et al (1972) suggested the type I cell to be the stimulus transducer, causing subsequent depolarisation of the afferent nerve terminal, possibly by release of a transmitter. Stimulation of the superfused carotid body in vitro induces release of substances capable of exciting a second carotid body, downstream, even if the stimulated carotid body has been previously denervated (Eyzaguirre et al, 1965; Eyzaguirre & Zapata, 1968). It is not clear, however, whether release of such substances from type I cells occurs at synaptic sites, or is associated with the extracellular space. 'Non-synaptic' release of transmitter does not preclude its affecting

the nerve terminals, since they could respond to a general concentration of a transmitter, rather than to a localised high concentration within a finite synaptic cleft (Torrance, 1968). Paintal (1969) raised serious doubts that release of transmitters could be responsible for the effect seen in these 'Loewi-type' experiments; he estimated that the amount of ACh required to be released to cause the effects seen would be some 50% of the carotid body's total mass (~2 mg in the cat). The alternative explanation offered by Paintal was that changes in PO_2 of the superfusate would account for the excitation of the second carotid body.

The cellular component appears to be necessary for the generation of afferent activity, since chemosensitivity is lost if the cells are destroyed. Verna and co-workers (1975) destroyed type I cells and the carotid body in the rabbit by cryocoagulation, and showed the recovery of chemosensory activity in the regenerating sinus nerve terminals only after they re-established connections with surviving type I cells. This prerequisite of nerve contact with type I cells has also been shown following sinus nerve crush (Zapata et al 1976); in these experiments chemoreception did occur when nerve to cell contacts were relatively few, and immature, indicating that the normally high number of such contacts represents a large safety factor, being greater than would appear strictly necessary. Smith and Mills (1979) observed that the regenerated sinus nerve, following extirpation of both carotid bodies, in cats, did not respond to the usual adequate chemoreceptor stimuli, but only to direct electrical stimulation.

Chemosensitivity can be induced in 'foreign' (vagal or superior laryngeal) nerve fibres implanted into the carotid body (de Castro,

1951; Zapata et al, 1969). It is not possible, though, to determine whether the type I cells are the primary transducer, or whether they 'condition' nerve endings to become chemosensitive (Eyzaguirre et al, 1977), only that their functioning is obligatory to chemoreception.

Ischaemia studies (Nishi et al, 1981) also suggest the requirement of type I cells for chemoreception. One hour after ischaemia, induced by arterial occlusion, signs of destruction were seen in type I cells, whilst nerve endings appeared intact. There was a specific lack of response to asphyxia and to cyanide, yet the responses to ACh and HCl were retained. After prolonged ischaemia (2-3 hours) there was also destruction of nerve terminals, and a decrease in responsiveness to all forms of stimulation.

1.4.4 Are the nerve terminals the chemoreceptors?

Evidence that the nerve terminals themselves are the chemotransducers is slender; Mitchell and co-workers (1972) found that some nerve fibres in a neuroma formed from the cut end of the sinus nerve of the cat show normal chemoreceptive properties in the supposed, but by no means certain, absence of type I cells. The reinnervation of the carotid body itself, or of ectopic chemoreceptor tissue (Matsuura, 1973) cannot be ruled out, especially since 12-18 months elapsed between the original surgery and the testing of chemoreceptor activity. Similar claims have been made for comparable experiments in the rabbit by Kienecker's group (1978), and by Tan et al (1981), where allowance was made for the criticisms levelled against the earlier experiments in the cat, but species differences cannot be ruled out.

1.4.5 Are the sensory nerve endings spontaneously active?

The idea that the regenerative region of the nerve terminals is spontaneously active has been propounded by Biscoe (1971). The pattern of chemoreceptor discharge is compatible with a Poisson (i.e. random) distribution, which might be explained by a fluctuation in excitability of small nerve endings consequential to thermal noise (hence spontaneous activity) or a fluctuation in excitability caused by random release of quanta of transmitter (?from type I cells). Theoretical considerations of fibre dimension and geometry, K^+ depolarisation predicted from the Nernst equation ($Em = RT/F.\ln[K^+]_o/[K^+]_i$, assuming permeability to K^+ ions only), and the activity of Na^+/K^+ ATPase and oxidative phosphorylation processes (Biscoe, 1971) have been used to argue that nerve endings are sensitive to local oxygen levels. Experimental support might be sought in the reinervation experiments (Kienecker et al, 1978; Tan et al, 1981), but other confirmation (e.g. the geometry and size of the nerve endings) is largely negative.

McDonald and Mitchell (1975a,b), assuming the nerve endings to be chemosensitive, ascribed to the type I cells the function of 'interneurones' in an inhibitory feed back loop, releasing dopamine (to inhibit the sensory terminals) after they themselves have been excited by a transmitter released from the sensory nerve terminals during natural stimulation. Such a theory is speculative, in as much as the contents and synaptic nature of the vesicles occuring in the afferent fibres are unknown, and the vesicles are presumed to be 'synaptic' only from morphological comparison with other structures.

1.4.6. 'Mechanical' theory.

Paintal (1977) reviewed the common features of sensory receptors: a non-neural element, sensitive to the magnitude and rate of change of the stimulus, which provides a measure of the stimulus, undergoes physical or dimensional changes, transmitting information mechanically to the 'generator' region of the sensory nerve ending. This induces a local change in the membrane potential ('generator potential' e.g. stretching of the crustacean stretch receptor causes deformation of the dendrites, and a reduction in membrane potential - Eyzaguirre & Kuffler, 1955), which reflects the intensity and duration of the stimulus; the signal is, however, rapidly attenuated, unless the stimulus is maintained or increased, resulting in the flow of more current, spreading to the adjacent region of the nerve fibre (e.g. the first node of the ending of a medullated fibre), leading to the initiation of an action potential at this 'regenerative' region, and which is propagated along the nerve fibre. The generator potential is graded in amplitude, depending upon the stimulus, but the action potential is 'all-or-none' in character, as is typical of any nerve fibre.

Because of the diverse chemical structures of the many substances that stimulate chemoreceptors, Paintal suggested that these all act non-specifically at the regenerative region of the sensory nerve terminals. His argument that CN^- is not a specific chemoreceptor stimulant, but also stimulates mechanoreceptors (Paintal, 1971), has not been upheld. McQueen (1980a) showed that low doses of ACh and other drugs with nicotinic properties would be unlikely to stimulate baroreceptors when injected i.c.; in addition he demonstrated that CN^- is a specific chemoreceptor stimulant in cats since it did not activate carotid baroreceptors at doses causing maximal chemoreceptor

stimulation.

In rejecting a requirement for chemical transmission, Paintal proposed that type II cells are chemosensitive, responding with a 'physical change' which results in excitation of the afferent nerve endings. A modification by Jones (1975) suggests the contraction of type II cells is effected by the release of ACh from type I cells.

1.4.7 'Cytochrome-metabolic' theory.

Mills and Jöbsis (1972) demonstrated the existence of an 'oxygen sensor' containing a cytochrome-a_3 with an unusually low affinity for oxygen, partial unsaturation occuring even at PO_2 of 140-150 mm Hg. The location of this O_2-sensor has been suggested to be the type II cells. Activation of the system would lead to the release of an excitatory 'transmitter' to act upon the afferent terminals. These authors have suggested that K^+ ions could leak out from the type II cells when their energy metabolism becomes compromised by hypoxia, and would be expected to depolarise nearby nerve endings, and perhaps type I cells also.

1.4.8 Theories based upon hydrogen ion activity.

Torrance's hypotheses (1976, 1977) on modulation of chemoreceptor activity as a function of intercellular acidity were formulated to explain the convergence of hypoxia and hypercapnia in afferent impulse generation in the same fibre. It was proposed that nerve endings respond to changes in local extracellular $[H^+]$, that pH in the finite space surrounding the nerve endings is controlled by H^+ inflow and HCO_3^- outflow to or from the glomeral blood, and that activity of the HCO_3^- pump controlling this exchange is dependent upon PO_2. This

theory relies upon the presence of extracellular carbonic anhydrase rapidly converting CO_2 to carbonic acid. Since benzolamide, a non-permeant inhibitor of carbonic anhydrase, was less effective than acetazolamide, which is freely permeant into cells, in slowing the chemoreceptor response to CO_2 (Hanson et al, 1981), the theory has been modified so that chemoreceptor discharge would be a function of intracellular pH.

Carbonic anhydrase has been located in type I cells (Lee, 1968) and in type II cells, (Becker et al, 1967). However, variations in cell pH during hypoxia in the carotid body in vitro have not been detected (García-Sancho et al, 1978). It is also known that CO_2 and H^+ ions stabilise the membrane of peripheral nerve fibres (Shanes, 1958), and hyperpolarise spinal motorneurones whilst increasing their input resistance (Marshall & Engberg, 1980); furthermore, barosensory nerve endings are depressed by low pH (Eyzaguirre & Zapata, 1968). It is possible that chemosensory afferents may have properties different to those of other sensory nerves. Indeed, intracellular recordings from the petrosal ganglion suggested that different Na^+ and Ca^{2+} currents underly the action potentials in chemosensory and barosensory fibres (Belmonte & Gallego, 1983). Of course, pH changes at the surface of the type I cells could also promote the release of transmitters.

1.5 Neurotransmitters in the carotid body.

Since type I cells contain many putative transmitter substances (see, for example, Eyzaguirre & Fidone, 1980), it is possible that one or more of these substances is an endogenous transmitter, released during natural or hypoxic stimulation and directly responsible for the initiation of neural discharge. Alternatively, the nerve endings

themselves may be spontaneously active, being depolarised by an oxygen-sensitive mechanism, and these transmitter substances could then act to modulate this spontaneous activity. Many substances have been proposed as possible chemotransmitters, none of which fully accounts for the whole body of experimental results obtained.

The fact that a large increase in blood pressure will abolish increased chemoreceptor discharge or reflex effects (Gernandt et al, 1945), and the failure of anaemic hypoxia to stimulate the carotid chemoreceptors suggests that chemosensory discharge is dependent less upon the amount of O_2 the blood supplies than upon the flow of blood through the carotid body, which supplies the chemoreceptors with something, the lack of which causes them to discharge, or alternatively, that the blood usually carries away some substance released in the carotid body, which is necessary for the excitation of sensory nerve endings.

Since chemoreceptor activity can persist after death, (perhaps for 30 minutes or more - Joels & Neil, 1963), the nerve fibres have minimal O_2 requirements for their functioning, and the apparent high O_2 consumption of the carotid body (e.g. 90 $\mu l\ g^{-1}\ min^{-1}$; Daly et al, 1954) is assumed to be a property of the (type I) cells (Joels & Neil, 1963). In the absence of oxygen these cells could release anaerobic metabolites or they could depolarise, releasing chemical transmitters that excite the sensory nerve endings. When there is no blood flow, as after death, or during intense vasoconstriction, the continued presence of metabolites or transmitters would maintain the discharge of chemoreceptors.

1.5.1 Acetylcholine as the 'essential' excitatory transmitter.

A long-favoured candidate as 'the' excitatory transmitter acting upon nerve terminals is ACh (e.g. Schweitzer & Wright, 1938; Landgren et al, 1952, 1954). However, hexamethonium may suppress reflex responses to injected ACh, without changing reflex responses to cyanide or hypoxia (Douglas, 1952), or it may reduce the chemoreceptor response to all forms of stimulation (Joels & Neil, 1962). McQueen (1977) showed that a number of agents affecting the chemoreceptor response to injected ACh had little or no effect upon sensitivity to hypoxia or CN^-; from the quantitative nature of his study, it appears doubtful that ACh is the primary agent involved in chemotransduction. Furthermore, Docherty and McQueen (1979) showed that in the rabbit exogenous ACh depresses chemoreceptor discharge. As the carotid body contains large amounts of cholinesterases (Hollinshead & Sawyer, 1945; Koelle, 1950), it seems unlikely that sufficient ACh would remain active, after death, to maintain the prolonged high level of discharge described above.

1.5.2 Catecholamines.

Evidence has been provided from both fluorescence microscopy studies and from autoradiographic studies of catecholamines in the carotid body to support the notion that these substances are stored in the type I cell DCVs (cf. Biscoe, 1971). Quantitative studies show little agreement as to how much of each CA is stored in the carotid body (see Table 1.2), although it is thought that dopamine (DA), and noradrenaline (NA) are present in greater abundance than adrenaline (ADR). These differences can result from differing sensitivities and selectivities of the methods used, or, perhaps more importantly, from

Table 1.2: Summary of results from quantitative studies of the catecholamine content of the carotid body (c.b.).

Author	Species	DA	NA	ADR	Units
Chiocchio et al (1966)	Cat	134 ± 23	81 ± 9	15 ± 3	ng/c.b.
Chiocchio et al (1967)	"	4.4 ± 0.4	2.4 ± 0.4	0.4 ± 0.1	$\mu g\ g^{-1}$
Zapata et al (1969)	"	204	98	24	ng/c.b.
Chiocchio et al (1971)	"	260	31 ± 51	*	ng/c.b.
Echeverría et al (1977)	"	56.5	40.9	3.4	$\mu g\ g^{-1}$
Mills et al (1978)	"	34 ± 2	161 ± 16	*	ng/c.b.
Mir et al (1982)	"	$^{+}$28 ± 5	$^{+}$123 ± 25	†	ng/c.b.
"	"	$^{\circ}$29 ± 6	$^{\circ}$137 ± 34	†	ng/c.b.
Hellström & Koslow (1975)	Rat	4.4 ± 1.5	2.9 ± 0.2	*	ng/pr c.b.
Hellström et al (1976)	"	4.6 ± 0.3	1.2 ± 0.04	*	ng/pr c.b.
Hellström (1977)	"	4.4 ± 1.2	1.2 ± 0.04	*	ng/pr c.b.
Hanbauer et al (1978)	"	2.6 ± 0.2	0.9 ± 0.1	*	ng/pr c.b.
Hansen & Christie (1981)	"	2.2 ± 0.2	1.6 ± 0.2	0.5 ± 0.07	ng/pr c.b.
Mir et al (1982)	"	$^{+}$2.5 ± 0.5	$^{+}$4.9 ± 0.8	n.d.	ng/pr c.b.
"	"	$^{\circ}$2.5 ± 0.6	$^{\circ}$0.5 ± 0.1	n.d.	ng/pr c.b.
Dearnaley et al (1968)	Rabbit	21.1 ± 3.6	*	*	ng/c.b.
Mir et al (1982)	"	$^{+}$33.0 ± 5.8	$^{+}$14.2 ± 3.5	n.d.	ng/c.b.
"	"	$^{\circ}$31.4 ± 4.9	$^{\circ}$6.9 ± 2.9	n.d.	ng/c.b.
Lishajko (1970)	Human (c.b. tumour)	8.2	2.0	3.2	$\mu g\ g^{-1}$
Mir et al (1982)	Ferret	16.1 ± 3.2	2.5 ± 1.0	n.d.	ng/c.b.
"	Guinea-pig	$^{+}$7.0 ± 1.7	$^{+}$36.8 ± 6.9	n.d.	ng/c.b.
"	"	$^{\circ}$7.9 ± 2.1	$^{\circ}$5.9 ± 2.4	n.d.	ng/c.b.

* = not reported; $^{+}$ = sympathetics intact; $^{\circ}$ = ganglionectomised c.b.; n.d. = not detected.
† = 5-9% of the value for DA.

the physiological status of the animal prior to the removal of tissue.

1.5.2.1 Synthesis & Precursors.

Catecholamines are synthesised from tyrosine and the rate limiting enzyme, tyrosine hydroxylase (TH), has been demonstrated in the carotid body of the rat, cat, and the rabbit (González et al, 1978, 1979a,b, 1981; Hanbauer, 1977; Hanbauer et al, 1977; Bolme et al, 1977). The activity of this enzyme persists after sympathectomy or sensory denervation (Fidone et al, 1980; Hanbauer & Hellström, 1978). TH-induction by prolonged or repeated exposure to hypoxia has been reported in rat carotid bodies (Hanbauer et al, 1977; González et al, 1979b), implying that chemosensory activity could regulate catecholamine synthesis.

Aromatic L-amino acid decarboxylase (DOPA decarboxylase) has been demonstrated indirectly in the carotid body of the cat (Chiocchio et al, 1971) and in the type I cells of the rat carotid body (Bolme et al, 1977), and it is concluded that type I cells have the capacity to synthesise DA.

Dopamine-β-hydroxylase (DβH) activity has been shown in the carotid body of the cat (Belmonte et al, 1977; Morgado et al, 1976), and also of the rat (Hanbauer, 1977), where activity is apparently unaltered by repeated exposure to hypoxia (Hanbauer et al, 1977). High levels of this enzyme are contained in the ganglioglomerular nerves, but the enzyme persists in the sympathectomised carotid body of the cat (Belmonte et al, 1977; Morgado et al 1976). Amine levels are little affected by chronic sympathectomy (3-7 days; Zapata et al, 1969), indicating that in the cat the major contribution to the NA content of the carotid body is from the type I cells. In the rat,

chronic sympathectomy (5-7 days) causes a reduction of some 50% in the NA content of the carotid body (Hanbauer & Hellström, 1978), and the distribution of DβH in rat type I cells is sparse (Bolme et al, 1977), from which it might be concluded that there is variation between species in the proportion of NA contributed by type I cells and by sympathetic nerve terminals. Since, in the rat carotid body, the NA content is also reduced by diethyldithiocarbamate-induced inhibition of DβH (Hellström, 1977), NA is at least partly synthesised in situ in addition to possible accumulation by uptake from the plasma.

Phenylethanolamine N-methyltransferase (PNMT) was not detected in type I cells of the rat carotid body (Bolme et al, 1977), which may explain the generally negative reports on the ADR content of the carotid body (see table 1.2).

[^{3}H]-tyrosine incorporation in the rabbit carotid body has been reported (Fidone et al, 1980, 1981), and prior exposure of animals to hypoxia resulted in increased formation of [^{3}H]-DA, but no significant increase in levels of [^{3}H]-NA.

It is unclear whether NA and DA are stored in the same DCV, in separate DCVs in the same cell, or in separate DCVs in different cells. Two varieties of type I cell have been described, on the basis of the electron density of their cytoplasm (Lever et al, 1959; Höglund, 1967; Grimley & Glenner, 1968; Chen et al, 1969; Morita et al, 1969; Abbott et al, 1972), or according to size and density of the DCVs contained in the cells (Kobayashi, 1968, 1975; Morita et al, 1969; McDonald & Mitchell, 1975b; Hellström, 1975), but there is no obvious correlation between the appearance and the CA content of any cell.

1.5.2.2 Pharmacological effects.

Many reports have appeared describing the effects of catecholamines upon ventilation and upon chemosensory discharge. The more salient features of the responses evoked by DA and by ADR or NA (and isoprenaline - ISO) are listed in tables 1.3, and 1.4 respectively.

It appears that there are some marked species differences in the effects upon the chemoreceptors of exogenous catecholamines. DA causes chemodepression in the cat, and ADR and NA transient chemodepression followed by an excitation. Both DA and NA appear to cause a stimulation of chemoreceptors in the dog, although low doses of DA also have chemodepressant effects in this species too (e.g. Bisgard et al, 1979). DA causes chemodepression in the rabbit in vivo (Docherty & McQueen, 1979), but in vitro studies are consistent with a predominant chemoexcitatory action (e.g. Eyzaguirre & Monti-Bloch, 1980).

'Biphasic' responses to NA have been variously reported, and varying explanations have been offered, most of which preclude an action at adrenergic receptors directly linked to the chemoreceptor complex. Respiratory excitation in response to NA or ADR is common, is blocked by α-adrenoreceptor antagonists such as phenoxybenzamine (Matsumoto et al, 1981; Yasuhara et al 1980) and is abolished by sectioning of the sinus nerves (Joels & White, 1968; Matsumoto et al, 1980a).

Excitatory effects of NA upon chemoreceptor discharge may be blocked by α-antagonists (e.g. Llados & Zapata, 1978b), and in the in vitro preparation, where vascular complications are said to be avoided, NA and ADR do not cause chemoexcitation (Eyzaguirre & Koyano, 1965; Zapata, 1975, 1977; Zapata et al, 1969) - or only very occasionally, and then in only a few preparations (Zapata, 1975, 1977).

Table 1.3: The effects of dopamine (DA) upon respiration or chemoreceptor activity.
(* denotes *in vitro* preparation; for other abbreviations see below).

Author	species	effects
Byck (1957)	Dog	Reflex hyperpnoea.
Young (1957)	Dog	Decreased ventilation
Jacobs & Comroe (1968)	Dog	Reflex hyperpnoea.
Black *et al* (1972)	Cat	Decreased ventilation.
Sampson (1972)	Cat	Chemodepression, antagonised by DBZ.
Mitchell & McDonald (1975)	Cat	DA effects antagonised by DHE.
Zapata (1975)	*Cat	Chemodepression, antagonised by SPIR, or DIB; repeated doses of DA lead to chemo-excitatory response instead of depression. ACh or CN^--evoked excitation reduced by simultaneous administration of DA.
Sampson *et al* (1976)	Cat	Chemodepression, antagonised by PHENT & DHE.
Nishi (1977)	Cat	Chemodepression, blocked with HAL or trifluperidol, but not by low doses of α-antagonists.
Sampson & Vidruk (1977)	*Cat & *Rabbit	Hyperpolarisation of nerve terminals.
Zapata (1977)	*Cat	Chemodepression, antagonised by DHE.
Zapata & Llados (1977)	Cat	DA effects antagonised by fluphenazine, with depression 'converted' to chemoexcitation.
Aminoff *et al* (1978)	Cat	DA effects antagonised by droperidol.
Docherty & McQueen (1978)	Cat	Chemodepression, antagonised by α-FLU.
Llados & Zapata (1978a)	Cat	DA chemodepression mimiced by apomorphine & amantidine; amphetamine or tyramine did not cause chemodepression. DA effects antagonised by HAL, SPIR, CHLORP, or PERPH, resulting in chemoexcitatory response to DA.
Llados & Zapata (1978b)	Cat	PHENB & DBZ not direct antagonists of DA effects, which are also resistant to PROP & DCI.
Welsh *et al* (1978)	Man	Indirect evidence of chemodepression.
Bisgard *et al* (1979)	Dog	Low doses - chemodepression; high doses - chemodepression preceded by chemoexcitation. Antagonism by HAL, DHE.
Docherty & McQueen (1979)	Rabbit	Chemodepression; antagonised by α-FLU.
Mishra *et al* (1979)	Rat	Chemoexcitation.
Bainbridge & Heistad (1980)	Man	Indirect evidence of chemodepression.
Cárdenas & Zapata (1980)	Cat	Reduced sensitivity to low [CN^-], but enhanced sensitivity to high [CN^-]
Cárdenas & Zapata (1980)	Rat	Depression of ventilation.

(Continued)

(Table 1.3 continued)

Author	species	effects
Eyzaguiurre & Monti-Bloch (1980)	*Cat	Receptor hyperpolarisation & reduced discharge, antagonised by HAL.
Monti-Bloch & Eyzaguirre (1980a)	*Rabbit	Receptor depolarisation & increased discharge, antagonised by HAL.
Lahiri & Nishino (1980)	Cat	DA blockade results in chemoexcitatory response to DA.
Lahiri et al (1980)	Cat	Decreased sensitivity to hypoxia & hypercapnia during DA infusion, but response potentiated after HAL.
Matsumoto et al (1980)	Rabbit	Chemoreflex depression of ventilation.
Zapata & Zuazo (1980)	Cat	Decreased ventilation.
Donelly et al (1981)	Cat	Enhanced basal discharge and sensitivity to hypoxia, but not to hypercapnia, after HAL.
Lahiri et al (1981a)	Cat	Chemodepression.
Nishino & Lahiri (1981)	Cat	Chemodepression.
Smatresk et al (1981)	Cat	DA depresses aortic chemoreceptors, but less so than the carotid chemoreceptors; effect reduced or reversed in severe hypoxia.
Folgering et al (1982)	Cat & Rabbit	Chemodepression, blocked by HAL & SPIR.

CHLORP: Chlorpromazine; DBZ: Dibenzyline; DCI: Dichloroisoprenaline; DHE: Dihydroergotamine; DIB: Dibenamine; α-FLU: α-Flupenthixol; HAL: Haloperidol; PERPH: Perphenazine; PHENB: Phenoxybenzamine; PHENT: Phentolamine; PROP: Propranolol; SPIR: Spiroperidol.

Table 1.4: Effects of adrenaline (ADR) noradrenaline (NA) and isoprenaline (ISO) upon respiration or chemoreceptor activity.
(* and † denote *in vitro* and decerebrate preparations respectively; for other abbreviations see below).

Author	species, drug and effect
Whelan & Young (1953)	Man — ADR,NA: Hyperpnoea.
Witzleb (1953)	Cat — ADR,NA: No detectable change in chemoreceptor activity.
Byck (1957)	Dog — ADR,NA: Reflex hyperpnoea.
Young (1957)	Cat — ADR: Increased ventilation, resistant to sinus nerve section.
Cunningham *et al* (1963)	Man — NA: Enhanced respiratory response to hypoxia.
Kuznetzov & Belen'kii (1963)	†Cat — ADR: Mild stimulation of respiration.
Biscoe (1965)	*Cat — ADR,NA,ISO: Chemoexcitation, blocked by pronethalol or DCI; hypoxia and ACh effects also blocked by DCI.
Eyzaguirre & Koyano (1965)	*Cat — ADR,NA: Chemodepression.
Heymans *et al* (1968)	Dog — Hypoxia and CN^- responses unaffected by α- or β-adrenoceptor blockade.
Jacobs & Comroe (1968)	Dog — NA: less effective than DA, but caused hyperpnoea; effect blocked by PHENT, but not by PROP.
Joels & Neil (1968)	Cat — ADR,NA (but not DA): Chemoexcitation.
Joels & White (1968)	†Cat — ADR: Increased ventilation, except when breathing 100% O_2 or after sectioning buffer nerves; increased chemoreceptor discharge. NA: Reflex hyperventilation, increased chemoreceptor discharge during NA infusion.
Zapata *et al* (1969)	*Cat — ADR,NA: No clear effects.
Black *et al* (1972)	Cat — NA: chemodepression, but inconsistently.
Heistad *et al* (1972)	Man — ISO: abrupt hyperpnoea.
Sampson (1972)	Cat — ADR,NA: chemodepression with secondary chemoexcitation; effects blocked by DBZ.
Zapata (1975)	*Cat — NA: No clear effect.
Flandrois *et al* (1977)	Dog — Adrenal medullectomy decreased plasma [ADR], but not plasma [NA], with resultant hyperpnoea, even under resting conditions.
Llados & Zapata (1978b)	Cat — NA: Chemoexcitation and increased discharge during infusion (blocked by α-antagonists); occasional chemodepression (enhanced by DIB, blocked by DA antagonists). ISO: Chemoexcitation only.
Wasserman *et al* (1979)	Cat — ISO: Chemoexcitation and reflex hyperpnoea, not abolished by 100% O_2.
Eclache *et al* (1979)	Man — Increased plasma [NA] produced hyperpnoea, reduced whilst breathing 100% O_2.
Winn *et al* (1979)	Rabbit — ISO: Hyperpnoea.
Yasuhara (1980) *et al*	Dog — ADR: Increased ventilation.

(Continued)

(Table 1.4 continued)

Author	species, drug and effect
Eldridge & Gill-Kumar (1980)	Cat	ISO: Intense hyperpnoea, blocked by section or cooling of carotid sinus nerve.
Zapata & Zuazo (1980)	Cat	NA: Increased discharge during infusion, with hypopnoea, not abolished by sinus nerve section.
Lahiri et al (1981a)	Cat	ISO: Intense hyperpnoea & increased discharge; chemoexcitation augmented by hypoxia and hypercapnia and partially blocked by PROP. Hypoxia and hypercapnia responses not blocked by PROP
Folgering et al (1982)	Cat, Rabbit	ADR,NA: Chemodepression (blocked by HAL) and chemoexcitation (blocked by PROP). ISO: Chemoexcitation only, partially blocked by PROP. Hypoxia response abolished by propranolol.
Gonsalves et al (1983)	Cat	ISO: chemoexcitation, blocked by PROP; hypoxia effect not blocked by PROP.

DCI: Dichloroisoprenaline; DIB: Dibenamine; HAL: Haloperidol; PHENT: Phentolamine; PROP: Propranolol.

It is widely accepted that the effect, in the carotid body *in situ*, stems from stagnant hypoxia or asphyxia caused by intense vasoconstriction (cf. Neil & Joels, 1963), and it is possible that α-adrenoceptors associated with the chemoreceptor complex are located on vascular smooth muscle cells controlling blood flow within the carotid body.

The situation may, in fact, be rather less simple since ISO is also a potent chemoreceptor stimulant (e.g. Gonsalves *et al*, 1983) which also causes reflex hyperpnoea (see table 1.4) through activation of β-adrenoceptors which would, presumably, be susceptible to activation by ADR and perhaps also by NA. Again, there is an apparent correlation of chemoreceptor excitation with vascular effects of the drug, in this case, the pronounced systemic hypotension evoked by ISO, and dichloroisoprenaline (DCI) and propranolol (PROP) block both ISO-evoked hypotension, and chemoexcitation *in vivo* (Llados & Zapata, 1978b); it is significant that ADR, which stimulates both β_1- and β_2-receptors (for the origins of the classification of adrenoceptors, which are the bases of contemporary nomenclature, see Ahlquist, 1948, and Lands *et al*, 1967a,b) as does ISO, does not cause chemoexcitation *in vitro* (Zapata, 1975; Zapata *et al*, 1969).

Biscoe (1965) reported that β-adrenoceptor blockade, which reduced chemoreceptor responses of the superfused cat carotid body *in vitro* to catecholamines, resulted also in depression of responses to hypoxia and ACh. In a comparable experiment Zapata *et al* (1969) could show no clear excitatory effect of catecholamines, and the depressant effects of DCI were considered to result from its local anaesthetic properties, rather than to its β-receptor blocking actions. Many β-blockers, because of their high lipid-solubility, may exert membrane stabilising effects, and allowance must be made for

the possible depression of chemoreceptor discharge as a result of local anaesthetic actions of some of these drugs.

More recently, Folgering et al (1982) reported that the β-adrenoceptor antagonists PROP and metoprolol (MET, a β-antagonist with 'less' membrane stabilising capacity than PROP) reduced or completely abolished the response of chemoreceptors to hypoxia. PROP, however, does not abolish reflex hyperpnoea in man (Heistad et al, 1972; Patrick & Pearson, 1978) or dog (Kontos & Lower, 1969), and Lahiri et al (1981a) could not demonstrate blockade of the hypoxic or hypercapnic response by PROP in the cat. Silva-Carvalho et al (1981) have more specifically demonstrated local anaesthetic blockade of chemoreceptor activity by PROP, by comparing the effects of administration of PROP or pindolol (a β-antagonist with considerably less membrane stabilising capacity than PROP), close to the carotid body, upon the respiratory changes elicited by injection of lobeline in the dog.

Mir et al (1984a) reported the presence of membranous β-adrenoceptors in homogenates of rat and rabbit carotid bodies, using [^{125}I]-cyanopindolol binding techniques. These receptors appear to be of the β_2-subtype, and are linked to the formation of cyclic adenosine monophosphate (cAMP). It was found that ISO, salbutamol (SAL), ADR, and histamine all significantly increased the cAMP content of the rat carotid body in vivo. NA, DA, and 5-hydroxytryptamine (5-HT) were ineffective, and the response to ISO was selectively blocked by β_2-, but not by β_1- antagonists. The precise location (and function) of these β-receptors remains to be determined.

DA-induced chemodepression is found consistently in cats, and the effect is blocked by a number of different classes of DA antagonist: butyrophenones, phenothiazines, thioxanthines, and

ergoloids. The effect is not mediated by vascular changes since it is also seen in the in vitro preparation (Zapata, 1975), where comparison of the weak effects of DA upon barosensory discharge also support the association of DA receptors with the carotid body chemoreceptors.

More recent evidence from receptor labelling studies (Dinger et al, 1981; Mir et al, 1984b), or pharmacological studies (McQueen, 1984) supports the designation of these receptors as D_2-receptors (Kebabian & Calne's classification, 1979). These DA-receptors could be associated with the sensory nerve endings (Okajima & Nishi, 1981), but perhaps also with the type I cells (Matsumoto et al, 1982) since DA, in low concentrations, can change the resting membrane potential of these cells.

Fitzgerald et al (1977) and Mir et al (1984b) failed to detect DA-sensitive adenylate cyclase in rabbit or cat carotid body homogenates, and so the predominant effects of DA appear to be mediated through DA receptor mechanisms that are independent of, or negatively linked to cAMP formation; the lack of positive linkage to adenylate cyclase is a distinguishing feature of Kebabian and Calne's D_2-receptor subtype.

In in vitro preparations sensory nerve endings are hyperpolarised in response to DA in the cat (e.g. Eyzaguirre & Monti-Bloch, 1980), but in the rabbit it is unclear whether DA induces depolarisation (Eyzaguirre & Monti-Bloch, 1980; Monti-Bloch & Eyzaguirre, 1980a) or hyperpolarisation (Sampson & Vidruk, 1977) of the sensory nerve endings.

The chemodepression elicited by NA in many of the experiments reported to date has been shown to be enhanced after α-adrenoceptor

blockade with dibenamine (Llados & Zapata, 1978b), or blocked by DA antagonists such as haloperidol (Folgering et al, 1982), and is perhaps a consequence of non-specific activation of DA inhibitory receptors. There are indications that NA exhibits some affinity for mammalian brain dopamine receptors (Seeman et al, 1978).

In dogs and in cats pre-treated with drugs blocking the depressant effects of DA, chemoexcitation or reflex hyperpnoea is sometimes observed in response to DA. The effect is not blocked by dibenamine (Zapata & Zuazo, 1982) and does not then represent a non-specific action of DA at α-adrenoceptors (which may be activated by high doses of DA - Goldberg, 1972).

'Primary' DA-induced chemoexcitation (that is, the excitation that does not require prior application of blockers of DA depressant effects before it becomes detectable) in dogs was reported to be blocked by D-tubocurarine (but not by succinylcholine or gallamine - Bisgard et al, 1979). DA-evoked hyperpnoea in cats pre-treated with spiroperidol persisted after D-tubocurarine administration (Zapata & Zuazo, 1982), and hyperventilation evoked by DA in dogs was resistant to blockade with hexamethonium. The DA excitatory effect is, therefore, unlikely to be mediated by nicotinic ACh receptors known to be present in the carotid body (Eyzaguirre & Zapata, 1968).

Excitatory DA receptors appear to have no affinity for apomorphine (which stimulates receptors mediating the depressant effects of DA) since apomorphine does not elicit chemoexcitatory effects after blockade of inhibitory effects (Llados & Zapata, 1978a; Zapata & Zuazo, 1982) in cats.

Zapata (1975) demonstrated an apparently labile character of the DA-receptors mediating depressant effects; following repeated injec-

tions of DA at short intervals a delayed excitatory response emerged following the chemodepression, and successive injections of DA ultimately resulted in a purely excitatory response to this catecholamine. The inhibitory effects of DA were blocked by spiroperidol and by dibenamine; neither antagonist, however, caused blockade of the excitatory component of the response to DA. DA-induced chemoexcitation following administration of spiroperidol is unchanged after the additional administration of haloperidol (Zapata & Zuazo, 1982). DL-DOPA was also found capable of inducing chemoexcitation, but not chemodepression (Zapata, 1975) and it has a rather poor affinity for brain DA receptors (Seeman et al, 1978).

In the cat carotid body in vitro the inhibitory effects of DA may partially, or even wholly, counteract the chemoreceptor stimulating properties of simultaneously applied CN^- or ACh, and in those preparations where an excitatory response to DA is observed, the excitatory effects of the catecholamine and of simultaneously administered chemoreceptor stimulants are additive (Zapata, 1975). Nishi (1977) has shown the reduction of chemoreceptor excitation in response to hypoxia or NaCN by the simultaneous application of DA.

1.5.2.3 Depletion Studies.

Depletion of catecholamine stores in cats by prolonged exposure to reserpine reduced chemoreceptor responses to ACh (Koppanyi & Cowan, 1962) or to hypoxia (Cowan & Greene, 1965), completely abolished chemoreceptor activity (Comroe, 1964), or had no effect upon chemoreceptor activity or responsiveness (Zapata et al, 1969; Nishi, 1977). It has been reported that chronic treatment with 6-hydroxydopamine (6-OHDA) causes a reduction in catecholamine fluorescence in the rat carotid

body (Lassman & Böck, 1972; Hess, 1975a). Others have failed to observe this effect upon catecholamine fluorescence (Hansen & Ord, 1978), or content (Hellström, 1977). Zuazo and Zapata (1978) observed no changes in chemoreceptor responses to CN^- and hypoxia in the cat up to seven hours after intracarotid injection of 6-OHDA, but, unfortunately, they made no determination of the degree of catecholamine depletion (if any) caused by this manouevre. Heymans et al (1968) showed that the response of chemoreceptors to hypoxia persisted after reserpine treatment in dogs.

1.5.2.4 Release of catecholamines.

It has been reported that the number of DCVs in type I cells following hypoxia is either unchanged (Zapata et al, 1969) or increased (Al-Lami & Murray, 1968) in the cat, unchanged in the hamster (Chen et al, 1969), increased in the rabbit (Møller, et al, 1974), and reduced in the rat (Hoffman & Birrel, 1958; Blümcke et al, 1967). It is uncertain whether a reduced number of DCVs reflects a specific effect of hypoxia, or results from pathological changes, nor is it certain that the number of DCVs reflects the amine content of the cell.

The amounts of DA, ADR, and NA measured in the cat carotid body before (see table 1.2) and after hypoxic stimulation in vivo and in vitro appear not to differ according to Zapata et al (1969). Mills and Slotkin (1975), however, found the total amount of NA and ADR in the cat carotid body was significantly reduced following hypoxia (8-40%, for 60-90 minutes), and that the extent of the reduction was dependent upon both the magnitude and the duration of the hypoxic stimulus; sectioning the sinus nerve attenuated hypoxia-induced

depletion of carotid body NA and ADR. Electrical stimulation of the sinus nerve also causes depletion of DCVs in the hamster type I cell; the effect may be blocked with atropine (200 mg kg^{-1}; Yates *et al*, 1970), although the effects associated with such a large dose of antagonist may not be specific or meaningful.

DA, but not NA, levels have been reported to be significantly decreased in rat carotid bodies after the animals had been exposed to hypoxia, and the effect was seen whether or not the carotid sinus nerve had been previously sectioned (Hellström *et al*, 1976). DA-depletion was partially relieved by administration of atropine, but preferentially stimulated by the muscarinic agonist methacholine. It was proposed that release of DA from type I cells is triggered by the activation of muscarinic receptors on the cell membrane by ACh. The source of this ACh was suggested to be either intrinsic cholinergic neurones or the type I cells themselves. Further studies (Hanbauer and Hellström, 1978) confirmed the preferential depletion of DA over NA by hypoxia and, further, showed that it represents an increased release of DA, rather than an increased turnover of DA; in their study chronic sinus nerve section caused an increase in the NA content of the carotid body. González and Fidone (1977) have suggested that in the rabbit too chemoreceptor stimulation leads to the release of DA.

Sampson *et al* (1975) demonstrated an increase in the intensity of formaldehyde-induced fluorescence in type I cells of the carotid body of normal cats, following stimulation of the cut end of the sinus nerve, and a reduction in fluorescence intensity in cats pre-treated with MK-486, a DβH inhibitor. This has been taken to indicate that efferent discharge in the sinus nerve may stimulate the synthesis and release of catecholamines.

1.5.2.5 Hypotheses on the role of catecholamines.

The most marked and most consistent effects of catecholamines are those obtained with DA. Osborne and Butler (1975) proposed a model suggesting that DA is released from type I cells and depresses activity in otherwise spontaneously-active sensory nerve endings. When the chemoreceptors are stimulated DA secretion is reduced and the nerve endings are 'disinhibited' so that afferent nerve activity increases. The hypothesis also suggests that a chemical transmitter, perhaps ACh, is released from the sensory nerve endings, to act upon type I cells, further reducing DA secretion as a consequence of a positive feedback loop.

The prominent feature of this hypothesis is that chemosensory activity is not the result of activation of otherwise quiescent nerve endings by an excitatory transmitter, but is due to disinhibition of spontaneously active nerve endings. Krammer (1978) has lent support to the model, and suggests that the release of NA, as a result of stimulation of a specific population of type I cells, decreases the otherwise continuous release of DA, which normally tonically inhibits the nerve endings. These hypotheses tend to rely upon the diminution of DA secretion during hypoxic stimulation; evidence already cited suggests that DA release is, in fact, either unchanged, or increased during hypoxia. It would be difficult to reconcile the potent excitatory effects of DA in the dog without invoking the principle of 'species variation', although it has also been shown that the effects of low doses of DA in the dog are depressant.

McDonald and Mitchell (1975a) have suggested that the sensory nerve endings are themselves chemosensitive whilst the type I cells are dopaminergic 'interneurones' exerting local control. They proposed

that afferent nerve endings are coupled to type I cells by 'reciprocal synapses', and that when stimulated the nerves release a transmitter which promotes DA release from the type I cells, which in turn acts upon the sensory nerves (negative feedback), to suppress afferent activity. This hypothesis has the advantage that DA secretion would be expected to increase during stimulation; Zapata (1975) supports the concept that endogenous DA may modulate afferent chemoreceptor activity, acting as a 'brake'. No role for carotid body NA has been postulated in this theory, which Krammer (1978) views as an oversight on the part of McDonald and Mitchell, who took no account of the possible separation of type I cells into two distinct populations (McDonald & Mitchell, 1975b). Krammer herself has suggested that the cytochrome mechanism of Mills and Jöbsis (see above) could reside in one class of type I cell which appears to be presynaptic to the other class of type I cell; the cytochrome-containing cells would release NA during hypoxia, by an O_2-dependent rise in cytosol Ca^{2+} (cf. Douglas, 1968, 1974). The role of the second series of type I cells would be that of dopaminergic interneurones, forming the final common pathway for modulation of the activity of the sensory nerve endings.

1.5.3 Indoleamines - 5-hydroxytryptamine.

1.5.3.1 Location within the carotid body.

The 5-hydroxy- derivative of tryptamine (5-HT) functions as a neurotransmitter, eliciting responses in both central nervous tissue and at locations in peripheral organs, by actions at 'tryptamine' (Gaddum, 1953) receptors.

Hamberger et al (1966) identified 5-HT-containing cells in the

human carotid body using fluorescence microspectrophotometry. Quantitative estimations of the 5-HT content of the cat and rat carotid bodies have since been made, and some of the results are listed in table 1.5, with the levels of DA determined in the same experiments shown for comparison.

Like catecholamines, 5-HT may be stored in DCVs in the carotid body type I cells of cats (Chiocchio et al, 1967) and hamsters (Chen et al, 1969), although Grönblad and Korkala (1977) have suggested that 5-HT is located in the interlobular mast cells of the (rat) carotid body.

1.5.3.2 Pharmacological studies.

Intravenous injection of 5-HT was reported to stimulate ventilation in the dog (Page, 1952), a phenomenon attributed to carotid chemoreceptor stimulation (Douglas & Toh, 1953; Comroe and Mortimer, 1964), an action of 5-HT upon the C.N.S. (Heymans & van den Heuval-Heymans, 1953), or to the initiation of cardiac and pulmonary reflexes (Schneider & Yonkman, 1954). Ventilatory responses to 5-HT are species-dependent: it is normally a respiratory stimulant in dogs and rabbits, but in the cat excitation is commonly preceded by apnoea (Schneider & Yonkman, 1954). Ginzell and Kottegoda (1954) showed that the secondary excitation of respiration in the cat was abolished by section of the carotid sinus nerve.

McCubbin et al (1956) recorded afferent chemoreceptor activity in the sinus nerve of the dog and found that i.c. injection of 5-HT caused a marked stimulation of chemosensory discharge, suggesting that the ventilatory response to 5-HT in the dog, at least, is due to direct chemoreceptor stimulation. This was repeated and confirmed by

Table 1.5: Examples of quantitative estimations of the 5-HT and DA content of the carotid body (c.b.).

Author	Species	5-HT	DA	Units
Chiocchio et al (1967)	Cat	6.9 ± 0.3	4.4 ± 0.4	$\mu g\ g^{-1}$
Chiocchio et al (1971)	"	10*	260	ng/c.b.
Hellström (1977)	Rat	7.5 ± 1.4	28.9 ± 7.9	pmol/pr c.b.
McQueen & Mir (1984)	"	~18	~23	pmol/pr c.b.

* estimated value assuming cat c.b.weighs ~2 mg (Jones, 1975)

Black et al (1972), but in cats they found a consistent immediate apnoeic to 5-HT, which was not abolished by sinus nerve section.

Eyzaguirre and Koyano (1965) found that 5-HT normally caused slight depression of the cat carotid chemoreceptors in vitro, although in a single experiment it caused chemoexcitation. Black et al (1972) reported that, in the in situ cat carotid body, injection of 5-HT could cause depression, excitation, or no change in spontaneous activity.

The most comprehensive pharmacological study, to date, of the effects of 5-HT upon chemoreceptors is that of Nishi (1975). He reported a brief, intense stimulation of chemoreceptors, in vivo, followed by a depression or block of activity lasting several seconds. Repeated injections of 5-HT at short intervals led to a rapid diminution of the response: this desensitisation or tachyphylactic effect may be the cause of the inconsistency of responses reported in this species. According to Nishi the effects of 5-HT upon carotid sinus baroreceptor afferents were qualitatively similar to the effects upon chemoreceptor activity, and he suggested that the indoleamine exerts non-specific effects upon sensory nerve endings and is thus unlikely to function as a chemical transmitter in the process of carotid body chemotransduction.

(+)-Lysergic acid diethylamide (LSD), methysergide, and gramine, which are (non specific) 5-HT antagonists, were found to block the vascular responses to 5-HT, but not the chemoreceptor responses (Nishi, 1975), suggesting that chemoreceptor and vascular responses are mediated by different 5-HT receptor types. It should be noted that LSD has agonist properties at some 5-HT receptor sites, and Nishi did note an excitatory effect of LSD itself upon chemoreceptors.

There appear to be no reports, as yet, of the release of 5-HT from carotid body cells during chemoreceptor stimulation, nor is there much information concerning the presence and distribution of the enzymes of 5-HT metabolism in the carotid body, although monoamine-oxidases (catabolising catecholamines and 5-HT) are present (Lee & Mattenheimer, 1964). Since 5-HT is present in the carotid body, and can promote changes in afferent activity, further studies seem warranted to determine whether it might have some role in the processes of chemotransduction.

1.5.4 Polypeptides.

A number of polypeptides have been demonstrated in the nervous system which fulfil the criteria demanded of neurotransmitters (for reviews, see Lord et al, 1977; Iversen, 1979; Hökfelt et al, 1980; Snyder, 1980), and much has been made of the ability of these peptides to act both 'discretely' as neurotransmitters as well as more generally as hormones (as is also the case with NA).

The application of immunohistochemical and radioimmunoassay techniques has led to the demonstration of enkephalin (ENK)-like, substance P (SP)-like, and vasoactive intestinal polypeptide (VIP)-like immunoreactivity in the carotid body (Lundberg et al, 1979a; Wharton et al, 1980; Cuello & McQueen, 1980; Fitzgerald et al, 1981). A major limitation of these techniques is the frequent lack of specificity of the immune reaction, so that a more or less broad spectrum of related peptides is demonstrated, rather than an individual substance.

Hökfelt (1979) has defined several distinguishing features of the 'classical' small molecular weight neurotransmitters and the peptides

(Table 1.6). Peptides are produced only on the ribosomes of the cell soma, probably in the form of larger precursor molecules (Gainer et al, 1977), from which the active peptides are liberated by enzymatic cleavage. High concentrations of the small classical transmitters are maintained by local synthesis in the nerve endings, active reuptake of released transmitters from the synaptic space, and by axonal transport of transmitters or precursors from the soma (Iversen, 1967).

No reuptake mechanisms appear to operate for neurotransmitter peptides, and their replenishment in the nerve terminals is dependent upon axonal transport. The apparently inefficient (or conservative) mechanism of peptide turnover may be reflected in the dynamics of the synaptic events they mediate (Bloom, 1977). The effects of peptides are often subtle, but of prolonged duration, and it could be that peptides are largely responsible for 'resetting' the resting membrane potential, rendering a cell more or less susceptible to excitation or inhibition by other (classical) transmitters.

1.5.4.1 Opioid peptides - methionine and leucine enkephalins.

Extracts of porcine brain yielded a morphine-like substance that mimicked the effects of morphine upon electrically induced smooth muscle contraction (Hughes, 1975), with equal susceptibility to blockade with the opioid antagonist naloxone (NAL). This substance contained two pentapeptides, methionine enkephalin ([Met]enk) and leucine enkephalin ([Leu]enk), differing only in the amino acid residue at the carboxyl terminal (see Appendix 2). These peptides were later isolated also from calf brain (Simantov & Snyder, 1976), and radioreceptor assay demonstrated that they were localised in nerve endings (Simantov et al, 1976), and thus might be neurotransmitters.

Table 1.6. Differences between classical small neurotransmitters and putative peptide neurotransmitters (cf. Hökfelt, 1979).

	Classical transmitters	Peptide transmitters
Molecular weight:	~200	~600 (enkephalins) >1000 (others)
Synthesis:	Enzymatic.	Ribosomal.
Site of synthesis:	Nerve endings (soma, axon).	Cell soma.
Storage:	Small vesicles.	Large vesicles.
Supply for release:	Local synthesis, reuptake, axonal transport	Axonal transport, cleavage from precursors.
Inactivation:	Reuptake, enzymatic, diffusion.	Enzymatic, diffusion.

Ca^{2+}-dependent release of ENK in response to depolarisation in brain tissue has been described (e.g. Smith et al, 1976; Iversen et al, 1978).

The five residues constituting [Met]enk are contained within the 91 amino acid residues of β-lipotropin, isolated from the pituitary by Li (1964). A variety of β-lipotropin fragments, including β-endorphin (β-END), and all containing [Met]enk possess opiate activity (e.g. Ling et al, 1976). Hughes (1978) considered β-END to be a 'prototype' opioid peptide, interacting equally with a variety of opioid receptors, whilst [Met]- and [Leu]- enk, which are more susceptible to rapid proteolytic inactivation, act more selectively at specific receptor subtypes. It is possible that β-END would function primarily as a neuroendocrine agent, whilst enkephalins may be more effective as discrete neurotransmitters.

Mechanisms of the cellular effects of opioids have been studied in a number of preparations. [Leu]enk increases the threshold for spike-generation in cultured mouse spinal neurones (Barker et al, 1978, 1980),and the effects of the peptide upon voltage-dependent ion conductances were antagonised by NAL. Similarly, evoked calcium spikes in the cell bodies of cultured avian sensory neurones are diminished in the presence of opioids (Mudge et al, 1979), and also by catecholamines; both opioids and catecholamines were inhibitors of the release of SP from these cells. In the frog spinal cord in vitro [Met]enk hyperpolarises primary afferent terminals, depressing the amplitude of both spontaneous and evoked depolarisations (Nicoll et al, 1980).

The slow inhibitory post-synaptic potentials (IPSPs) set up in sympathetic ganglion cells, following pre-synaptic activation, are not antagonised by NAL, even though [Met]enk can hyperpolarise ganglion

cells by a NAL-sensitive mechanism (Wouters & van den Bercken, 1979; 1980). In this case the pharmacological effect of the peptide is, presumably, different to the physiological processes of IPSP generation, which are more likely to result from the activity of DA or ACh, both of which can also hyperpolarise ganglion cells, the effects of each, and the generation of IPSPs being antagonised by DA or ACh antagonists (cf. Kuba & Koketsu, 1978). [Met]enk can depress slow IPSPs, coincidently with its hyperpolarising action at the pre-ganglionic component of the synapse, and it has been suggested (Wouters & van den Bercken, 1979; 1980) that the peptide suppresses release of a transmitter mediating inhibition.

Studies of the guinea-pig myenteric plexus *in vitro* (North *et al*, 1979) have revealed a NAL-sensitive hyperpolarising response to bath application of opioid peptides. Focal application of the peptides to the region of the cell body produced a depolarisation, associated with an increase in membrane resistance, which was not antagonised by NAL. When the site for iontophoresis was a neural process, NAL-sensitive hyperpolarising responses were recorded which could lead to a diminished release of other transmitters from the cell.

Despite the lack of knowledge concerning the mechanisms that underlie opioid actions upon nerve cells, ligand binding studies have suggested the involvement of more than one type of receptor site (μ,δ,κ,σ), with differing affinities for the opioid peptides that may be found in the intact organism (e.g. Paterson *et al*, 1983; see also Section 8).

ENK-like immunoreactive material has been detected in cells, but not in the nerves of the cat carotid body (Lundberg *et al*, 1979a; Wharton *et al*, 1980), with [Met]enk being some three to four times

more abundant than [Leu]enk (Wharton et al, 1980).

The two forms of ENK appear to be equipotent depressants of chemosensory discharge in cats when injected into the carotid artery in vivo (McQueen & Ribeiro, 1980), but it has been reported (Monti-Bloch and Eyzaguirre (1980b) that [Met]enk increases chemosensory activity in the in vitro preparation. Excitatory responses to injected ACh or CO_2-equilibrated Locke solution were slightly potentiated during i.c. infusions of [Met]enk (McQueen & Ribeiro, 1980), but cyanide evoked chemoexcitation was reduced. This study was not sufficiently wide in its scope for more to be said than that opioid peptides appear to be able to modify evoked responses, as well as spontaneous discharge.

Low doses of NAL greatly reduce or abolish chemodepression evoked by END, but part of the depressor response to ENK is resistant to NAL blockade (McQueen & Ribeiro, 1980; 1981b), and ENK may exert actions at δ-receptors (which are less susceptible to naloxone) whilst β-END may have greater activity at μ-receptors (which are more readily blocked by NAL - see Lord et al, 1977; Pert et al, 1980). Chemoexcitation in response to ENK or END has been seen after treatment with NAL, which could be due to activity at NAL-insensitive (κ?) receptors, or to non-specific effects. Pokorski and Lahiri (1981) showed an enhanced chemoreceptor response to hypoxia in NAL-treated cats, implying that endogenous opioids normally exert some tonic depressant action upon the chemoreceptors. NAL had no obvious effect upon responses to hypercapnia, although the ventilatory increase in response to CO_2 was enhanced after the antagonist, suggesting a more important mediation of ventilatory responses to CO_2 at central sites.

In parts of the nervous system ENK (and β-END) can affect Ca^{2+}

uptake leading to a reduction of transmitter release (Guerrero-Munoz et al, 1979). DA, a 'prime' inhibitor of chemosensory discharge, continues to depress spontaneous discharge in the presence of NAL (McQueen & Ribeiro, 1980), and the depressant effects of ENK are not appreciably affected by the DA antagonist α-flupenthixol (McQueen, 1981).

ENK-like immunoreactivity has been demonstrated in the carotid body type I cells (Hansen et al, 1982) where it appears that opioid peptides are co-stored with NA, as in the adrenal medulla (Schultzberg et al, 1978). It is not known whether opioid receptors in the carotid body would respond to circulating opioids released from the adrenal glands (Costa, 1980; Viveros et al, 1980) or by sympathetic nerves (Wilson et al, 1980; Konishi et al, 1981), and the conditions under which carotid body opioids are released remain to be determined.

1.5.4.2 Substance P.

To date no certain physiologically important role of SP has been proven. This vasodepressor substance occurs in gut tissue and in neural tissue, particularly the dorsal root ganglion cells, where Ca^{2+}/Mg^{2+}-dependent, K^{+}-evoked release of SP has been demonstrated (Otsuka & Konishi, 1976). Of particular interest when studying carotid body chemoreception is the finding that release of SP from sensory terminals in the spinal cord depends upon activation of more slowly conducting non-myelinated or thinly myelinated Aδ and C fibres (Jessell et al, 1979; Yaksh et al, 1980). A role as a sensory transmitter in the mediation of pain reflexes is popularly accepted (cf. Gobel & Binck, 1977); opiates block the release of SP in spinal cord (Jessell & Iversen, 1977; Mudge et al, 1979) and this could perhaps

contribute to opiate analgesia. The possibility of interactions between SP and ENK is further suggested by the finding of SP- and ENK-containing neurons in juxtaposition in the raphe nuclei, ventral tegmental area, the septum, and the amygdala (see Hökfelt et al, 1980).

The mechanisms of action of SP remain uncertain. Iontophoresis or perfusion with SP has been shown to alter the membrane potential of myenteric neurones (Katayama et al, 1979; Katayama & North, 1980), commonly resulting in depolarisation of the cell and an increase in membrane resistance. The effect is voltage dependent, reversing at about -95 mV, suggesting that decreased K^+ conductance contributes to the depolariasation. This pharmacological effect of SP has yet to be correlated with the characteristic synaptic events, slow depolarisations, seen, for example, in myenteric neurones (Wood & Mayer, 1978, 1979; Johnson et al, 1980a).

Application of SP to frog spinal cord motoneurones appears to cause an increase in membrane conductance and a direct depolarisation of about 5mV, which is insufficient to attain threshold for the generation of action potentials (Nicoll, 1978). In the presence of SP subthreshold motoneurone excitatory post-synaptic potentials (EPSPs), generated by stimulating synaptic inputs from sensory fibres, were able to trigger action potentials, which may be a consequence of the partially depolarised state of the motoneurone in the presence of this polypeptide.

At the frog neuromuscular junction (Steinecker, 1977) brief exposure to SP causes a transient decrease in presynaptic quantal content, resulting in a decrease in end-plate potential; there then follows a period of several hours during which both quantal content

and end-plate potential are increased. Together these results suggest that SP can facilitate transmission between sensory and motor neurones and at the neuromuscular junction.

Quantitative studies have shown that pancreatic acinar cells release K^+ ions in response to a rise in intracellular [Ca^{2+}] evoked by SP and by muscarinic or α-adrenoceptor agonists (Putney et al, 1980). Supramaximal doses of any two agonists did not produce additive effects and Putney (1977) proposed that K^+ efflux is dependent upon activation of an homologous population of Ca^{2+} channels, regardless of the receptor specificity of the activating agonist applied. Comparing the kinetics of the binding of radiolabelled ligands Putney and co-workers (1978) found that the same biological response resulted from the occupation of about 15000 α-adrenoceptors, 1800 muscarinic receptors, or just 200 SP receptors, implying that the amplification of the effects of SP binding is far in excess of that for the other agonists. This equates well with the proposition, already cited, that peptidergic neurotransmission is 'conservative' in nature.

Cuello and McQueen (1980) demonstrated the presence of SP-like immunoreactive material in both cells and nerve endings of the carotid body. When injected i.c. SP has been shown to cause an initial slight chemodepression followed by a dose-related increase in discharge; these effects were more delayed, longer lasting, and less intense than those evoked by ACh or DA injections, and the excitation appeared to correlate with a rise in $PaCO_2$, which may, in turn, have been consequential to SP-induced changes in bronchiolar tone or cardiac output (McQueen, 1980b). Responses to ACh and DA were reduced during SP infusion, whereas responses to NaCN were potentiated

(McQueen, 1980b). Some of these effects could have been due to blocking or modulatory actions of SP at nicotinic ACh receptors (cf. similar actions of SP on Renshaw cells - Ryall & Belcher, 1977). SP did not overcome or prevent chemodepression caused by i.c. infusion of [Met]enk (McQueen & Ribeiro, 1981b). Monti-Bloch and Eyzaguirre (1980b) reported that SP reduced spontaneous discharge in the cat carotid body in vitro, and similarly in rabbits and mice (unpublished observations, see Eyzaguirre & Fidone, 1980).

No studies appear yet to have established whether there are SP-receptors (see Section 9) in the carotid body itself, or whether SP is at any time released from sites in the carotid body.

1.5.4.3 Vasoactive intestinal polypeptide.

VIP is a highly basic 28 amino acid peptide which shares many sequential similarities and metabolic or gastrointestinal properties with other 'intestinally'-derived peptides such as glucagon and secretin (Said & Mutt, 1969, 1970; Mutt & Said, 1974). Although originally isolated from extracts of porcine duodenum, this peptide has been found in neurones throughout the gastrointestinal tract, and in fibres of the peripheral nervous system innervating the lungs, pancreas, and urogenital tract (Fahrenkrug, 1979). In all such areas these fibres are associated with smooth muscle close to mucosal surfaces, glandular epithelia, and around blood vessels, the pattern of distribution equating well with the peptide's established pharmacological effects such as smooth muscle relaxation, vasodilatation, and enhanced secretion from exocrine glands (Said, 1980).

VIP or related peptides have been found in the CNS of many mammalian species (Fahrenkrug, 1979, 1980) where the distribution

of VIP-like immunoreactivity appears to be concentrated in forebrain areas (cf. Fahrenkrug et al[§], 1978; Loren et al, 1979; Emson et al, 1979). In the CNS there also seems to be an association between VIP and the pial and cerebral blood vessels suggesting that VIP-neurones may control local or regional blood flow (Larsson et al, 1976, Lindvall et al, 1978).

Ca^{2+}-dependent release of the peptide from rat hypothalamic slices in vitro and from synaptosomal preparations made from rat cerebral cortex, hypothalamus, and striatum has been reported (Giachetti et al, 1977; Emson et al, 1978). The presence of VIP, that can be released in response to stimulation, has been shown in the small-diameter primary afferent fibres of the cat spinal cord (Go & Yaksh, 1980). In association with the peripheral nervous system, VIP has been measured in venous blood, the amount increasing when the parasympathetic nerves to the gastrointestinal tract are stimulated (Fahrenkrug et al, 1978; Bloom & Edwards, 1980a).

Coexistance of VIP and ACh in the postganglionic fibres that innervate salivary, sudiferous, and nasal mucosal glands has been reported in the cat (Lundberg et al, 1979b, 1980, 1981), and both substances can be released by stimulation of the postganglionic nerves (Bloom & Edwards, 1980b; Lundberg, et al 1980, 1981); ACh causes an atropine-sensitive increase in secretion and VIP induces vasodilatation close to the site of release of secretory products. The simultaneous infusion of ACh and VIP markedly potentiates both vasodilatation and secretory responses in the salivary gland (Lundberg et al, 1980), the effects of the two substances applied together being more than additive, suggesting a synergistic function.

Membrane binding sites for VIP have been demonstrated in guinea-

[§]Fahrenkrug & Schaffalitzky de Muckadell

pig and rat brain (Robberecht et al, 1978, 1979; Gammeltoft et al, 1980; Taylor & Pert, 1979); in brain slice and synaptosomal preparations VIP has been shown to stimulate cAMP formation (Quik et al, 1978, 1979; Borghi et al, 1979; Kerwin et al, 1980), although the distribution of VIP binding sites is poorly correlated with the distribution of VIP-stimulated cAMP formation. This stimulation of cAMP formation is unaffected by phenoxybenzamine, propranolol, α-flupenthixol, or naloxone, implying that VIP-sensitive adenylate cyclase is independent from the receptors activated by other neurotransmitters that stimulate formation of cAMP.

Application of VIP to neurones in the cerebral cortex, hippocampus, and spinal cord causes depolarisation and neuronal excitation; many cortical cells responding to VIP react also to ACh, but the effects mediated by the peptide are resistant to blockade by atropine (Phillis et al, 1978).

Since femtomolar amounts of VIP excite rat hippocampal preparations (Dodd et al, 1979; Dingledine et al, 1980), this peptide might possess extraordinary potency as a neurotransmitter.

Few studies of the effects of VIP upon chemoreceptor activity have been reported. Fitzgerald et al (1981) showed a slight elevation of spontaneous discharge during the close arterial infusion (5-25 μg in 0.5 ml over 10s) of VIP in the cat in vivo, and McQueen and Ribeiro (1981a) confirmed that a rise in discharge occurs which is sustained throughout a 5 minute infusion of VIP (0.5 μg min^{-1}) into the carotid artery. The response to injected VIP was variable - low doses (<0.25 μg) causing chemodepression, higher doses (>0.25 μg) chemoexcitation (McQueen & Ribeiro, 1981a); infusion of the peptide reduced chemoexcitation evoked by ACh, NaCN, or CO_2-equilibrated Locke

solution, and also chemodepression evoked by [Met]enk.

1.6 Scope of the present study.

The coexistence of peptides with classical transmitters (and ATP) is now largely accepted (cf. Burnstock et al, 1979), as is the occurance of receptors to different agents in the same tissue. In particular, coexistance has been found in the adrenal medullary cells (e.g. Schultzberg et al, 1978), and, more importantly, in the carotid body (Lundberg et al, 1979a; Hansen et al, 1982).

The presence of neuropeptides and classical neurotransmitters in the carotid body, together with their apparent importance in neurotransmission in other parts of the nervous system, prompted the present investigation of their effects upon carotid body chemoreceptors, and this thesis includes experiments where the effects of the two types of transmitter have been studied together. Attempts have been made to clarify the actions of catecholamines, in particular NA, since there is much dispute as to the effects of these transmitters and their significance. Use has been made of newer and more selective agonists and antagonists for determining the receptor types responsible for the observed affects upon chemosensory activity, and as far as possible the effects of these drugs upon the chemoreceptor responses to physiological stimulation by hypoxia or hypercapnia (the natural governing parameters of carotid chemoreceptor activity) have also been studied.

SECTION 2

METHODS AND MATERIALS

SECTION 2.

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Experiments were performed on 11 adult male cats (weight range: 2.0-3.9 kg; mean weight [± s.e.m.]: 2.95 ± 0.17 kg; median weight: 2.9 kg) and 74 adult female cats (weight range: 2.2-4.0 kg; mean weight [± s.e.m.]: 2.89 ± 0.05 kg; median weight: 2.9 kg), and 13 rabbits (male, New Zealand white strain; weight range: 2.5-3.5 kg; mean weight [± s.e.m.]: 3.05 ± 0.13 kg; median weight: 3.2 kg).

2.1 Anaesthesia.

56 cats were anaesthetised by intraperitoneal (i.p.) injection of pentobarbitone sodium (Sagatal, 60 mg ml^{-1}), 42 mg kg^{-1}, supplemented every one to two hours with 6-12 mg injected i.v. The other 29 cats were anaesthetised with α-chloralose, 1% in 0.9% saline, injected intravenously (i.v.) at a dose of 60-70 mg kg^{-1}, following induction of anaesthesia by inhalation of 5% halothane in oxygen. Supplementation of the anaesthetic was rarely necessary, but if required, a further 40 mg was injected i.v. In five cats which were difficult to manage, or distressed by handling, anaesthesia was induced with halothane prior to injection of pentobarbitone.

Rabbits were anaesthetised with pentobarbitone sodium (Sagatal, 60 mg ml^{-1}, diluted 1:2 with 0.9% saline), 30-40 mg kg^{-1}, administered through an ear vein, and supplemented as necessary.

2.2 General.

Experimental procedures employed were common to both cats and

rabbits. Following a midline incision in the ventral aspect of the neck, a tracheal cannula was inserted below the level of the larynx. Both femoral arteries were cannulated with nylon catheters (external diameter 1.34 or 1.65 mm). One catheter was connected to a blood pressure transducer (Bell and Howell, 4-442), the signal from which was displayed on an oscilloscope (Tektronix, 5103N), and simultaneously recorded on a pen-recorder (Electromed, MX6) and on one channel of an FM tape recorder (Tandberg 100; frequency response d.c. to 1250 Hz). The second catheter was used for the withdrawal of blood samples for blood gas analysis, generally at hourly intervals, when arterial blood pH, PO_2, and PCO_2 were measured using a Radiometer gas monitor (BMS 3, with PHM 71 meter). Arterial samples were also analysed after steady state had been achieved following a change in the composition of inspired gases, or during some drug infusions, when a minimum interval of 3 mins between samples was found to be most practical.

A femoral vein was cannulated (as above) for the administration of anaesthetic supplements, neuromuscular blocking drugs, drugs soluble only in large volumes of solvent or in an acid medium, and drugs with known membrane-stabilising properties.

Heart rate was monitored with an E.C.G. amplifier, the signal being relayed to a loudspeaker and to one channel of the oscilloscope, and in some experiments the output from the E.C.G. amplifier was also recorded on one channel of the tape recorder.

Rectal temperature was monitored continuously, and maintained at 38 ± 0.5°C by an electric heating blanket.

2.3 Respiration and neuromuscular blockade.

Animals were artificially ventilated with room air by a respiratory pump (S.R.I.), operating at 18-19 strokes per minute for cats, and 25 strokes per minute for rabbits. Stroke volume was adjusted so as to maintain $PaCO_2$ in the region of 30-35 mm Hg, and end tidal CO_2, continuously monitored by an infra-red CO_2 analyser (Med 1A, Grubb Parsons), at 4-5%. Under such conditions, PaO_2 was generally 90-100 mm Hg. The inspired gas was room air, but animals could be made hyperoxic or hypoxic by ventilating with specific mixtures of oxygen and nitrogen. The proportion of each gas was regulated using calibrated flow-meters, with the gases being mixed in a reservoir prior to administration to the animal. A 'standard hypoxia test' was carried out by ventilating the animal with 10% O_2:90% N_2 for 4 minutes, followed by 100% N_2 for 1 minute, and recovery on 100% O_2 for 2 minutes. Hypercapnia could be induced by adding carbon dioxide to the inspired gases, and by adding carbon dioxide to a mixture of 25-30% oxygen in nitrogen, $PaCO_2$ was raised with no significant change in PaO_2 from that measured during air-breathing.

Once a suitable recording of chemoreceptor activity was obtained, spontaneous respiration was arrested by paralysing the animal with gallamine triethiodide (Flaxedil, 40 mg ml^{-1}), 3 mg kg^{-1} i.v., the dose being repeated as required, usually when supplementing the anaesthetic. Neuromuscular blockade was employed to prevent movement of the nerve on the recording electrodes as a result of spontaneous muscle contraction, or muscle movements induced by close-arterial injection of ACh, and to prevent spontaneous or drug-induced respiratory movements which would alter arterial blood gas tensions. Chemoreceptor discharge shows no marked variation when comparing

results obtained in the spontaneously breathing, and the artificially ventilated animal, nor does gallamine triethiodide appreciably affect chemoreceptor activity evoked by NaCN or ACh (see McQueen, 1977).

2.4 Dissection.

The carotid bifurcation was approached through the ventral incision in the neck, and dissected free of surrounding tissues. A nylon catheter (external diameter 0.63 or 0.75 mm) was inserted into the lingual artery, and advanced until its tip lay in the common carotid artery, 1.5-2.0 cm caudal to the carotid bifurcation. In some experiments a second such catheter was inserted into the same common carotid artery via the superior thyroid artery, the tip being advanced caudally to terminate some 2.0-3.0 cm from that of the lingual catheter. The dead-spaces of the lingual and thyroid catheters were ~0.1 and ~0.2 ml respectively. The patency of either catheter, and the effectiveness of its location could be confirmed by the injection of a low dose of a chemoreceptor stimulant such as NaCN.

The lingual catheter was used for intracarotid (i.c.) administration of drugs to the carotid body by injection or infusion; the thyroid catheter was used for infusion of drug solutions whilst allowing the injection of a second drug via the lingual catheter simultaneously, thus permitting the combined effects of the two drugs to be studied.

2.5 Recording of carotid sinus nerve activity.

The carotid sinus nerve ipsilateral to the catheterised common carotid artery was identified distally to the carotid body at the site of its junction with the glossopharyngeal nerve and cut centrally.

Exposed tissues were covered with warmed (37°C) mineral oil. The connective tissue outer sheath of the cut sinus nerve was stripped back towards the carotid body and the exposed nerve was placed on a small mirror to facilitate further dissection of fine filaments from the nerve using fine (26G) needles and watchmaker's forceps.

Extracellular afferent electrical activity in filaments of the nerve was recorded using bipolar platinum-iridium wire electrodes. The original signal was amplified by a factor of 10,000 by passage through an a.c. amplifier (Neurolog, Digitimer), displayed on the oscilloscope, and recorded on one channel of the tape recorder. The neural information being recorded on magnetic tape was constantly monitored by relaying the signal to the tape recorder back to another channel of the oscilloscope. The output from the amplifier was also passed through a voltage discriminator, the number of action potentials of a selected amplitude counted, and converted to an analogue signal proportional to the frequency of discharge, which was displayed on the pen recorder.

Chemoreceptor units were identified by their irregular pattern of discharge, their increase in frequency in response to: a) injection of NaCN (2.5-5.0 μg) into the ipsilateral common carotid artery; b) hypoxic ventilation with 10% O_2 in nitrogen; c) asphyxia evoked by tracheal occlusion, and by the substantial reduction in discharge frequency during hyperoxia (ventilation with 100% O_2). Single chemoreceptor units were identified from the constant shape and amplitude of the superimposed action potential; such units are characterised also by a delay of at least 7 ms between successive spikes (Biscoe and Taylor, 1963). Twenty four recordings of single chemoreceptor units were obtained in cats, and the majority of the other recordings were

of two to four units, of which it was often possible to count one unit separately. Recordings of chemoreceptor activity were obtained in nine rabbits, but none of these was of a single unit.

In all experiments on cats the sympathetic innervation of the carotid sinus region was eliminated by sectioning the ganglioglomerular nerves (a group of distinct nerve fibres projecting from the superior cervical ganglion to the carotid sinus region), in order to prevent reflex effects of sympathetic activity upon carotid sinus nerve discharge (e.g. Floyd and Neil, 1952; Eyzaguirre and Lewin, 1961). This procedure could not always be performed successfully in the rabbit, since the ganglioglomerular nerves tend to be much less discrete than in the cat, and these nerve fibres are also united with the sinus nerve close to its point of exit from the carotid body, so that sectioning of the sympathetic nerves also often results in denervation of the carotid body (Docherty, 1980).

2.6 Drug administration.

Intracarotid (i.c.) injections were generally made in a volume of 0.1 ml into the common carotid artery, and washed in with 0.2 ml of modified Locke solution which had been bubbled with 5% CO_2 in air at 37°C. Injections were made at the peak of the inspiratory phase of the respiratory cycle, and were accomplished within one respiratory cycle. A period of at least three minutes was allowed between injections, or longer if the drug under investigation was known to cause tachyphylaxis. Other drugs were injected i.v. in an appropriate volume, and the catheter flushed with 0.5-1.0 ml modified Locke solution, or 0.9% w/v saline. Infusions of drugs were made using a Braun 'Unita' pump, at a standard rate of 0.1 ml min^{-1}, and the amount

of drug infused per unit time was determined by altering the concentration of the solution infused.

Control injections of 0.1 ml modified Locke solution equilibrated with room air were made, and the catheter flushed with 0.2 ml Locke solution bubbled with 5% CO_2 in air at 37°C. These injections were made throughout each experiment, and served as a control to determine the effect upon chemoreceptor discharge of the drug vehicle, and of the injection procedure.

2.7 Drugs used.

Drugs were dissolved in modified Locke solution (millimolar composition: NaCl, 102.7; KCl, 5.6; $CaCl_2$, 1.6; Tris base, 49.5; pH 7.41 at 37°C) or, in the case of peptides and related substances, 0.9% w/v saline. Rauwolscine was dissolved in saline containing an equal weight of ascorbic acid, and domperidone, prazosin, and ICI 118,551 were dissolved in saline with the addition of a few drops of 0.1N HCl and the application of gentle heating. ICI 174,864 was suspended in half the final volume of saline and dissolved by addition of an equal volume of saline containing an equivalent amount of L-arginine. Prenalterol was obtained as a commercial solution ('Hyprenan') containing 1.0 mg ml^{-1}.

The drugs used, their sources, and details of their structures and molecular weights are to be found in appendices 1 and 2. Doses referred to in the text are those of the salt.

2.8 Data analysis.

The output of the tape channel containing the action potentials was passed through a Neurolog filter module (d.c.-10 KHz with 50 Hz

notch) and fed to a pulse height discriminator (W.P.I. model 120) permitting the counting of selected action potentials. The number of pulses occuring in 0.1 s intervals ('bins') was collected, and stored in the memory of a PET 32 K microcomputer (Commodore, 303 series), programmed to collect data over a specified period of up to 500 s. Having initiated the collection of data, a marker was set to indicate the point at which injection of a drug, or a change in inspiratory gas was made. Data were stored on floppy disc and subsequently analysed quantitatively using the microcomputer.

A computer-drawn plot of the frequency of action potentials as a function of time was displayed upon the computer screen, from which discharge was analysed in the 'control' (e.g. pre-injection) period, and either subjectively (i.e. during periods when discharge appeared to be increased or decreased with respect to control), or during defined intervals, such as the first five seconds after injection of a drug. When analysing the effects of injection of a drug, the control period was taken as approximately 15 s immediately preceding the injection. For injections of opioid peptides, which caused prolonged depression of discharge, the 'test' period chosen was the first 30 s after injection. In studying the effects of drug infusions, or of changes in inspired-gas composition, collection of data was started 30 or 60 s prior to onset of infusion or ventilation with a selected gas mixture, and discharge was then analysed in succesive 10, 15, or 30 s intervals.

The period 't' seconds, during which the computer was required to process discharge frequency was delineated using a moving cursor, which directed the computer to the memory-stored data represented upon the screen. For each test the following calculations were performed

by the computer:

i) Σx: the total counts (number of action potentials) occuring in the period t seconds, for control and for test periods. Units: 'counts'.

ii) $\bar{x}$ c.p.s.: the mean counts per second occuring in each period t seconds. Hence, $\bar{x}$ (control) = Σx(control)/t(control),

and $\bar{x}$ (test) = Σx(test)/t(test).

Units: 'counts per second' (c.p.s.).

iii) $\Delta\bar{x}$: the difference in the mean discharge occuring in test and control periods. Thus, $\Delta\bar{x} = \bar{x}$(test) - $\bar{x}$(control). Units: c.p.s.

iv) $\Delta\bar{x}\%$: derived by expressing $\Delta\bar{x}$ as a percentage change from the control mean discharge. Units: 'percent' (%).

v)$\Delta\Sigma x$: the 'absolute difference' of discharge from control levels, given by: $\Delta\Sigma x = \Sigma x$(test) - [$\bar{x}$(control) × t(test)]. Units: 'counts'.

vi) max: the maximum number of action potentials counted in any one second of a period t seconds. Units: 'counts'.

vii) Δmax: the difference between the maxima determined in the test and control periods. Units: 'counts'.

Of the above, iii, iv, and v may have negative values, where there is a depression of discharge, and i, ii, and vi may be equal to zero. Data integrated with respect to control (i.e. $\Delta\Sigma x$) take account of the two-dimensional character of responses, and both the magnitude, and the duration of the induced change in discharge are included in the analysis of the response (see McQueen, 1977). This parameter measures the increment or decrement in discharge in the test period, relative to the pre-injection discharge which, it is assumed, would have remained constant in the absence of any modifying effect of the drug.

Whenever possible, dose-response studies were performed and an appropriate parameter expressing the change from control was plotted against $\log_{10}$ dose. The range of doses over which the relationship was approximately linear was determined by inspection and a straight line fitted to the points in this part of the dose-response curve either by eye, or, for greater precision, and more usually, by the method of least squares. Where feasible, data from more than one experiment were pooled, and the overall mean of parameters such as ID_{50} or ED_{30} (drug doses causing respectively a 50% reduction, or a 30% increase in discharge when compared to pre-injection control discharge) were expressed as the arithmetic mean ($\bar{x} = \Sigma x_i/n$), ± standard error of the mean (s.e.m.). Pooled data for measurements of blood gas tensions were expressed similarly.

2.9 Analysis of hypoxia tests.

Discharge ($\bar{x}$ c.p.s.) was quantified in succesive 15 s periods both before (control) and after the onset of hypoxia. Discharge reached a 'plateau' or 'maximal level of discharge some 1.5 to 2 minutes after the onset of hypoxia. The rising phase of the response was generally a linear function of time, and a straight line was fitted to this part of the response using the least squares method. In any experiment the plateau discharge during steady-state hypoxia (10% O_2) before the administration of antagonists was arbitrarily defined as 100%, and the other levels of discharge measured at different phases of the test were expressed as a percentage of this value. The gradient of the line reflecting the rate of rise of chemoreceptor discharge was calculated in terms of % max s^{-1}, and was used as an index of chemoreceptor responsiveness. The time taken to achieve maximal (plateau) discharge

was also measured.

2.10 Statistical treatment of results.

In order to determine whether changes in discharge observed were statistically significant, responses were compared with appropriate controls, using the non-parametric Mann Whitney test or Student's t-test (when data could not be paired), and the Wilcoxon signed ranks test (when the number of pairs exceeded seven) or Student's paired t-test (when there were less than six pairs of results; cf. Colquhoun, 1971; Sard, 1978). The null hypothesis was rejected at the level of $P \leq 0.05$, and the difference between groups was considered to be statistically significant.

SECTION 3

STUDIES ON THE EFFECTS OF INJECTION OF NORADRENALINE AND SELECTIVE ADRENOCEPTOR AGONISTS AND ANTAGONISTS UPON CHEMORECEPTOR ACTIVITY IN THE CAT

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STUDIES ON THE EFFECTS OF INJECTION OF NORADRENALINE AND SELECTIVE ADRENOCEPTOR AGONISTS AND ANTAGONISTS UPON CHEMORECEPTOR ACTIVITY IN THE CAT.

3.1 Introduction.

Catecholamines are present in the cat carotid body (see Section 1) where, it has been suggested, NA is predominant (Mills *et al*, 1978). A more recent study (Mir *et al*, 1982) confirmed that NA is present in substantially greater amounts than DA in the carotid body of the cat and the guinea-pig (and perhaps in the rat), with the reverse distribution in the rabbit and the ferret. Evidence already cited suggests that in the cat a large amount of the total catecholamine content of the carotid body is derived from the cellular component rather than from the sympathetic nerve terminals. In the rat, at least, it appears that much of the NA is contributed by the sympathetic nerves (Mir *et al*, 1982). *In vitro* experiments have indicated that, in the absence of a functioning vasculature, the carotid chemoreceptors are not affected by the catecholamines ADR and NA (see Section 1), but both substances tend to cause chemoexcitation *in vivo*, which has generally been attributed to actions mediated by vascular α-adrenoceptors (e.g. Sampson, 1972; Llados & Zapata, 1978b), causing localised stagnant hypoxia (Joels & Neil, 1963). β-selective adrenoceptor agonists also cause chemoexcitation and according to some workers (Biscoe, 1965; Folgering *et al*, 1982) β-selective antagonists reduce the chemoexcitatory effects of ADR, NA, or ISO. Furthermore, Folgering and co-workers (1982) reported that the

response of chemoreceptors to hypoxia was greatly reduced or even abolished by β-antagonists, leading to the proposition that β-mediated catecholaminergic mechanisms are important, if not fundamental, to the processes of chemotransduction. It has been argued (Zapata et al, 1969) that the results of Biscoe (1965) could equally well be a result of non-specific nerve block consequent to membrane-stabilisation by the β-antagonist DCI.

Early reports often describe a transient depression of discharge preceding NA-evoked chemoexcitation, which has, more recently, been shown to be a 'non-specific' action of NA at DA receptors mediating chemodepression (e.g. Folgering, et al; 1982). It should be noted that, according to some earlier reports (cf. Section 1), chemodepression caused by DA was blocked (albeit non-specifically - Llados & Zapata, 1978b) by α-antagonists.

The present experiments were designed to determine which adrenoceptor subtypes could mediate the response of chemoreceptors to exogenously applied NA, and whether they might play some role in the chemoreceptor response to natural stimulation such as hypoxia. The following agonists and antagonists were employed, their commonly accepted selectivities for receptors (cf. Fig. 3.1) being indicated in parentheses: agonists - NA ($\alpha_1/\alpha_2/\beta_1$), phenylephrine (PHEN; α_1), oxymetazoline (OXM; α_2) - Starke et al, 1975a,b; isoprenaline (ISO; β_1/β_2 - Konzett, 1940 a,b; Lands et al, 1967a,b), dobutamine (DOB; β_1 - Tuttle & Mills, 1975), prenalterol (PREN; β_1 - Carlsson, et al, 1977b), and salbutamol (SAL; β_2 - Brittain et al, 1968); antagonists - corynanthine (COR) and rauwolscine (RAU; α_1 and α_2 respectively - Weitzell et al, 1979), metoprolol (MET; β_1 - Åblad et al, 1975), betaxolol (BET; β_1 - Boudot et al, 1979), ICI 118551 (β_2 - Bilski et al, 1979),

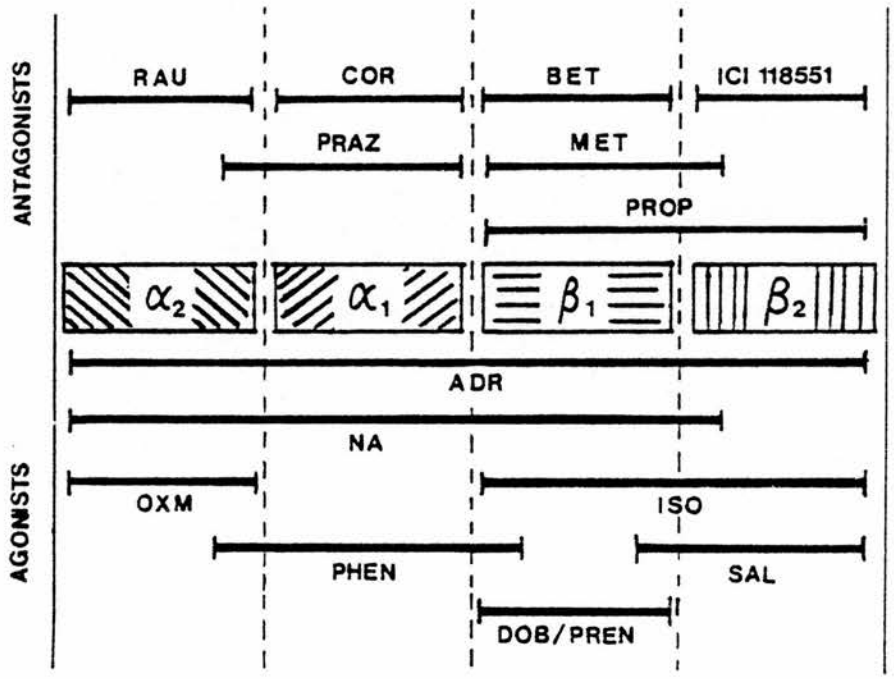


Figure 3.1. Schematic representation of the range of actions of agonists and antagonists at adrenoceptors. Width of bar gives an indication of the occurance or otherwise of an effect at that sub-class of receptor. (α_1: ADR≥NA,PHEN>>ISO; α_2: OXM≥NA>>ISO; β_1: ISO>NA≥ ADR>>SAL; β_2: SAL≥ISO>ADR>>NA - cf. Lees, 1981).

and domperidone (D_2-antagonist - Baudry et al, 1979).

3.2 Results.

3.2.1 Chemoreceptor responses to noradrenaline.

NA was injected into the common carotid artery in 53 cats, where the effects a wide range of doses (0.001-100 µg i.c.) were studied. No clear changes in chemoreceptor discharge were noted with doses of NA less than 0.5 µg; at higher doses the chemoreceptor response was characteristically biphasic. An initial depression of discharge was followed by a period of increased activity which, in some experiments, could be separated into two characteristically different phases (cf. Fig. 3.2). All components of the response were highly variable between experiments making quantitative analysis of chemoreceptor activity difficult and of questionable relevance (see Section 10).

3.2.1.1 Initial chemodepression.

An initial phase of chemodepression was observed in all experiments where the effects of injected NA were studied, and it was markedly greater than the artefactual depression of discharge occasionally associated with the injection of the drug vehicle (Locke solution - see Section 2). The threshold for chemodepression was about 1-5 µg, and the latency of onset very short, depression of discharge usually being seen to commence during or upon completion of the injection. The magnitude of this chemodepression was always less than that seen after i.c. injection of equivalent doses of DA (see Figs 3.3, 3.4, 3.5, 3.7). Depression of chemosensory activity was typically absolute for a few seconds, followed by a recovery period,

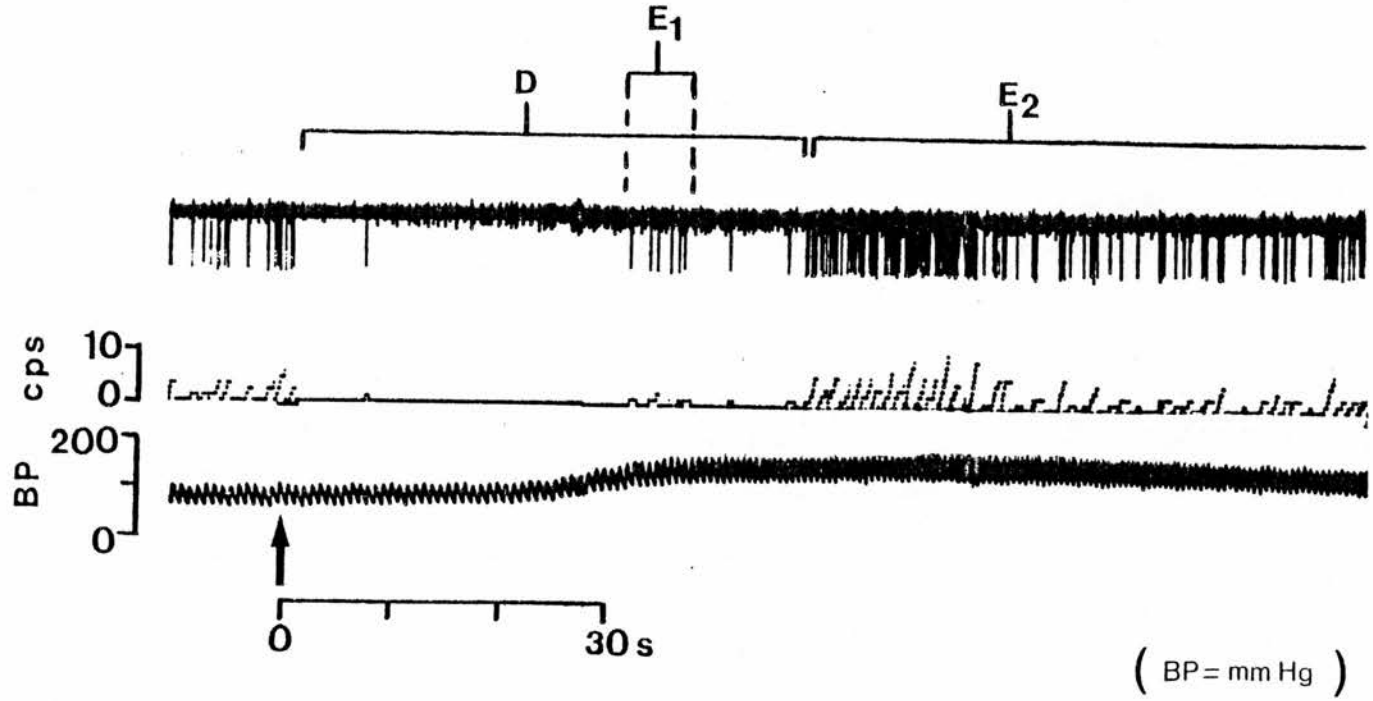
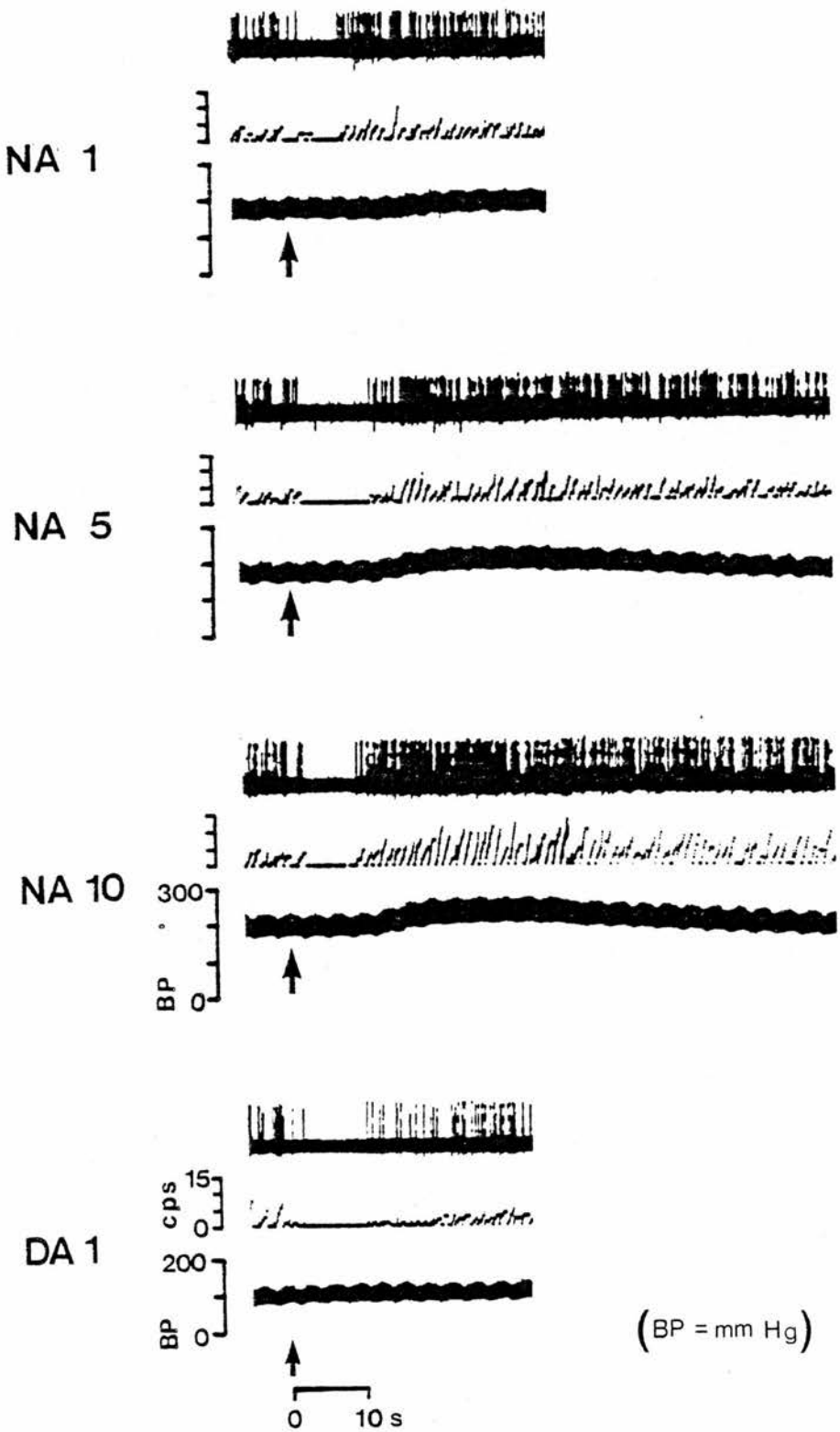


Figure 3.2. Neurogram showing the response of a single chemoreceptor unit to i.c. injection (at arrow) of NA, 10 µg. The ramped counter output beneath the neurogram shows the number of action potentials counted in successive 1 s intervals. Initial chemodepression (D) and delayed chemoexcitation (E_2) are clearly illustrated, as is the early, sharp (E_1) excitation, taking the form of a burst of action potentials occuring near the end of, but within, the period of chemodepression. The accompanying record of femoral arterial blood pressure, showing the hypertensive effect of the drug, demonstrates also the acceleration of heart rate in response to the drug, at the time of peak increase in blood pressure.

Figure 3.3. Effects upon chemoreceptor discharge and femoral arterial blood pressure of i.c. injections (at arrows) of NA: A, 1 µg; B, 5 µg; C, 10 µg, and D, DA, 1 µg. A ramped counter output below each neurogram shows the number of action potentials counted in successive 0.1 s intervals. In this experiment chemoexcitation was more apparent after injecting 5 and 10 µg NA and was temporally related to the changes in blood pressure.


NA 1
NA 5
NA 10
300
BP
0
DA 1
cps
15
0
200
BP
0
0
10 s
(BP = mm Hg)

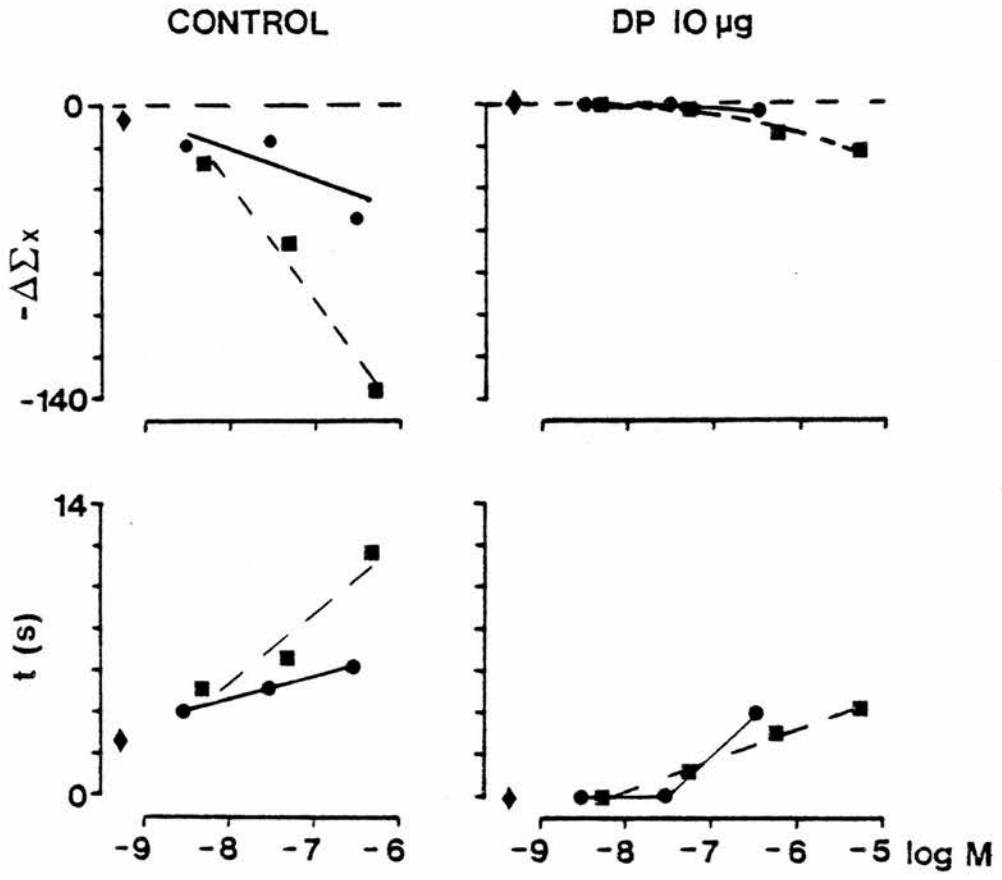
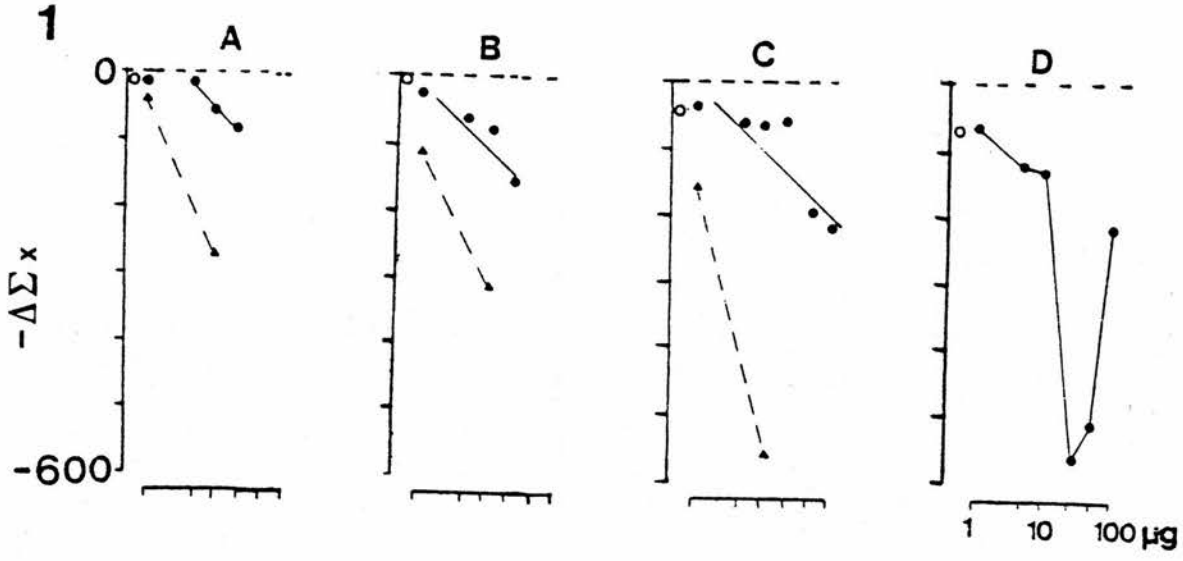


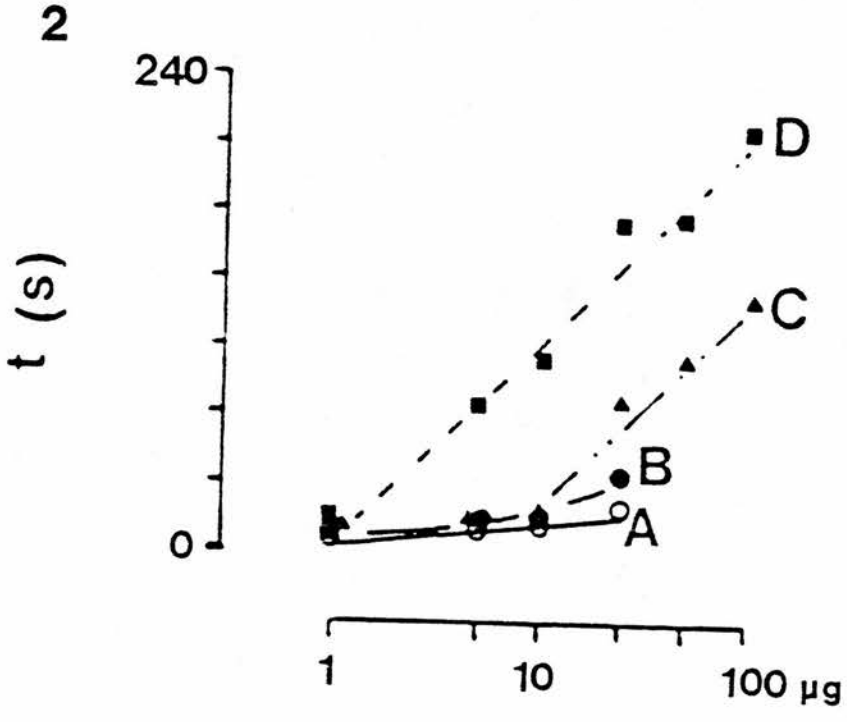
Figure 3.4. Analysis of data obtained in a single experiment. Magnitude ($\Delta\Sigma x$ - top panels) and duration (s - lower panels) of the chemoreceptor response to i.c. injection of NA (●---●) and DA (■ - ■), plotted against $\log_{10}$ dose, before (control) and after (DP 10) injection of domperidone, 10 µg kg^{-1} i.c.

♦ = effect of injecting Locke solution, 0.3 ml. Lines were fitted by eye to give a better assimilation of the data points.

Figure 3.5. (1) Responses of 2-3 chemoreceptor units to i.c. injection of NA (●) and DA (▲). Immediate chemodepression ($-\Delta\Sigma x$) plotted against dose, before (A) and after BET 0.1 (B), 1 (C), and 2.12 (D) mg kg^{-1}. Open circles denote effects of injection of Locke solution, 0.3 ml.

Chemodepression was potentiated after increasing doses of antagonist, as a consequence of the marked increase in duration of the response shown in part (2) of the figure, where duration (in seconds) of the chemodepressant effect is plotted against dose, before (A) and after BET 0.1 (B), 1 (C), and 2.12 (D) mg kg^{-1}. Lines were fitted to the data points by eye.


1
A
B
C
D
0
-600
$-\Delta\Sigma x$
1
10
100 µg


2
240
0
t (s)
D
C
B
A
1
10
100 µg

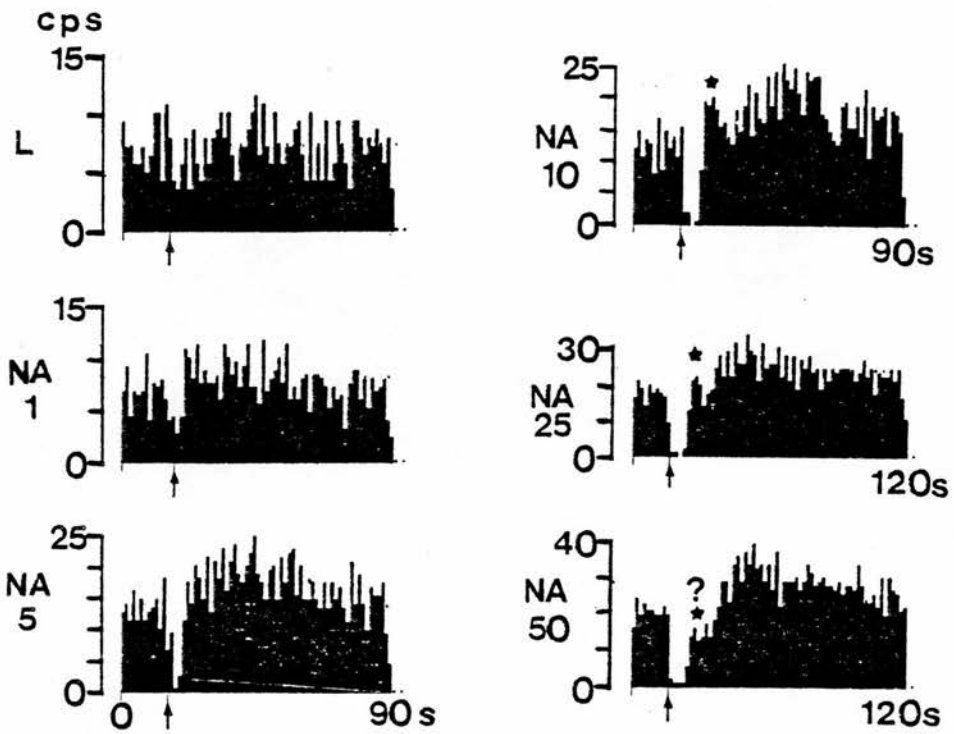


Figure 3.6. Computer-drawn plots of frequency of chemoreceptor discharge and the effects of i.c. injection (at arrows) of Locke solution, 0.3 ml, and NA 1, 5, 10, 25, and 50 µg. With higher doses of NA there were two distinct excitatory components to the response, the first of which (marked with an asterisk) became less distinct as the dose injected was increased. The tendency for a progressive increase in the basal rate of discharge should also be noted. Data are from a single experiment.

during which discharge frequency gradually increased towards pre-injection control levels. The magnitude of the overall response ($\Delta\Sigma x$) was commonly log-linearly related to the dose of NA, as was the duration of the effect (Fig. 3.4).

3.2.2 Chemodepressor effects of selective adrenoceptor agonists.

Much evidence from previous reports (cf. Section 1) suggested that NA-evoked chemodepression was mediated by DA-receptors, but it had also been suggested that α-antagonists could block DA-evoked chemodepression (e.g. Sampson 1972) or potentiate chemodepression caused by NA (e.g. Llados & Zapata, 1978b). The capacity of selective adrenoceptor agonists to elicit depression of discharge was examined to determine whether part of the chemodepression evoked by NA could be explained by effects at receptors other than DA-receptors, bearing in mind the possibility that chemodepression in response to NA could result from release of endogenous DA by NA (cf. Krammer, 1978).

Two α-selective agonists were studied. OXM (α_2; 0.1-200 µg i.c.) was virtually devoid of any chemodepressant activity (Fig. 3.11), causing no chemodepression in three experiments (60%) and transient, non-dose-dependent chemodepression in two (40%).

In nine experiments PHEN (α_1; 1 µg - 2 mg i.c.) only occasionally caused chemodepression; the effect was small but did show some evidence of being dose-dependent (Fig. 3.12).

Of the β-selective agonists examined (cf. Figs 3.12, 3.13) ISO (β_1/β_2; 0.01-100 µg i.c.) injected in seven experiments, SAL (β_2; 0.1 µg - 1 mg i.c.) in thirteen experiments, and PREN (β_1; 0.1 µg - 1 mg i.c. or (n=1), 5 mg i.v.) in three experiments did not cause depression of discharge other than a small inconsistent effect comparable to the

artefactual chemodepression occasionally associated with vehicle (Locke solution) control injections.

Dose response data for DOB, an hydroxylated aryl-alkyl derivative of DA with putative β_1-receptor selectivity, were obtained in one experiment, where injection of 1-50 µg i.c. caused mild chemodepression of almost constant magnitude and duration, but greater than that caused by injection of Locke solution (cf. Table 3.1). In a separate experiment i.c. injection of 100 µg DOB caused marked depression of discharge (~86% reduction from pre-injection control) lasting some two minutes.

3.2.3 Effects of antagonists upon NA-evoked chemodepression.

In eight out of nine experiments (89%) domperidone (DP; 10-100 µg kg^{-1}) blocked NA-induced chemodepression together with that evoked by DA (Fig. 3.4).

NA-evoked chemodepression was also partially reduced by the α_2-selective antagonist RAU (1 mg kg^{-1}; n=2 - see Figs 3.24 and 3.25), under which conditions the chemodepression caused by DA was also slightly reduced.

The β-antagonists BET and MET at high doses (e.g. 1 mg kg^{-1} or more) tended to potentiate both NA- and DA- evoked chemodepression. This potentiation was due to a marked prolongation of the period of chemosensory depression (cf. Figs 3.5, 3.14, 3.15, 3.18). Even when so potentiated, chemodepression was abolished by subsequent administration of DP or RAU.

3.2.4 Chemoexcitatory responses to NA.

Some degree of chemoexcitation followed chemodepression in all

Table 3.1: Effects of injection of dobutamine upon chemoreceptor discharge.

DOSE (µg)		ΔΣx	Duration (s)	$\Delta\bar{x}$%	$\bar{x}$(control) (c.p.s.)
DOB	1	-45	7.8	-54.8	10.5
	10	-44	6.5	-67.6	9.8
	25	-50	9.1	-57.3	9.4
	50	-47	7.8	-75.0	8.0
Locke 0.3 ml		-19	3.9	-69.9	6.7

experiments, although this part of the response was feeble in some recordings and intense in others. Close inspection of the response shows it to consist of two components which may be designated E_1 and E_2, E_1-type excitation preceding E_2-type. The excitatory component of the response to NA was analysed in terms of $\Delta\Sigma x$, $\Delta\bar{x}\%$, Δmax (see Section 2), and the duration of the response; E_1-excitation could not generally be analysed separately because of the narrow range of doses of NA that clearly elicited the response, and because it appeared to be obscured by chemodepression or E_2-excitation at higher doses.

E_1-excitation could be clearly identified in 35 (67.3%) of the recordings, but only at lower and mid-range doses of NA (Figs 3.2, 3.6). This phase of intense excitation was of short duration, lasting only a few seconds, and appeared to occur at the time when blood pressure and heart rate were actually increasing in response to NA. According to dose, E_1-excitation could appear as a transient burst of action potentials briefly interrupting the period of chemodepression and separate from E_2-excitation (e.g. Fig. 3.2); in other instances, this type of excitation preceded E_2-excitation as a distinct event, but with higher doses (Fig. 3.6) it became merged into, and indistinguishable from the more delayed E_2-excitation. When analysed in terms of $\Delta\Sigma x$, $\Delta\bar{x}\%$, or Δmax, E_1-excitation was inversely related to dose (Fig. 3.7).

E_2-excitation was observed in all recordings, although the magnitude of the response quantified by the parameter $\Delta\Sigma x$ was highly variable between experiments. The onset of this chemoexcitatory effect of NA appeared to be delayed until the establishment of equilibrium after the rapid rise in blood pressure, and the stabilisation of diastolic and systolic pressures (cf. Figs 3.2 and 3.3) at

Figure 3.7. Responses of two chemoreceptor units to i.c. injections of NA.

A: $\Delta\Sigma x$ for NA-evoked chemodepression and E_2-excitation (●), NA-evoked E_1-excitation (▲), and DA-evoked chemodepression (■) plotted against dose (note scale of the ordinate applied to E_1-excitation).

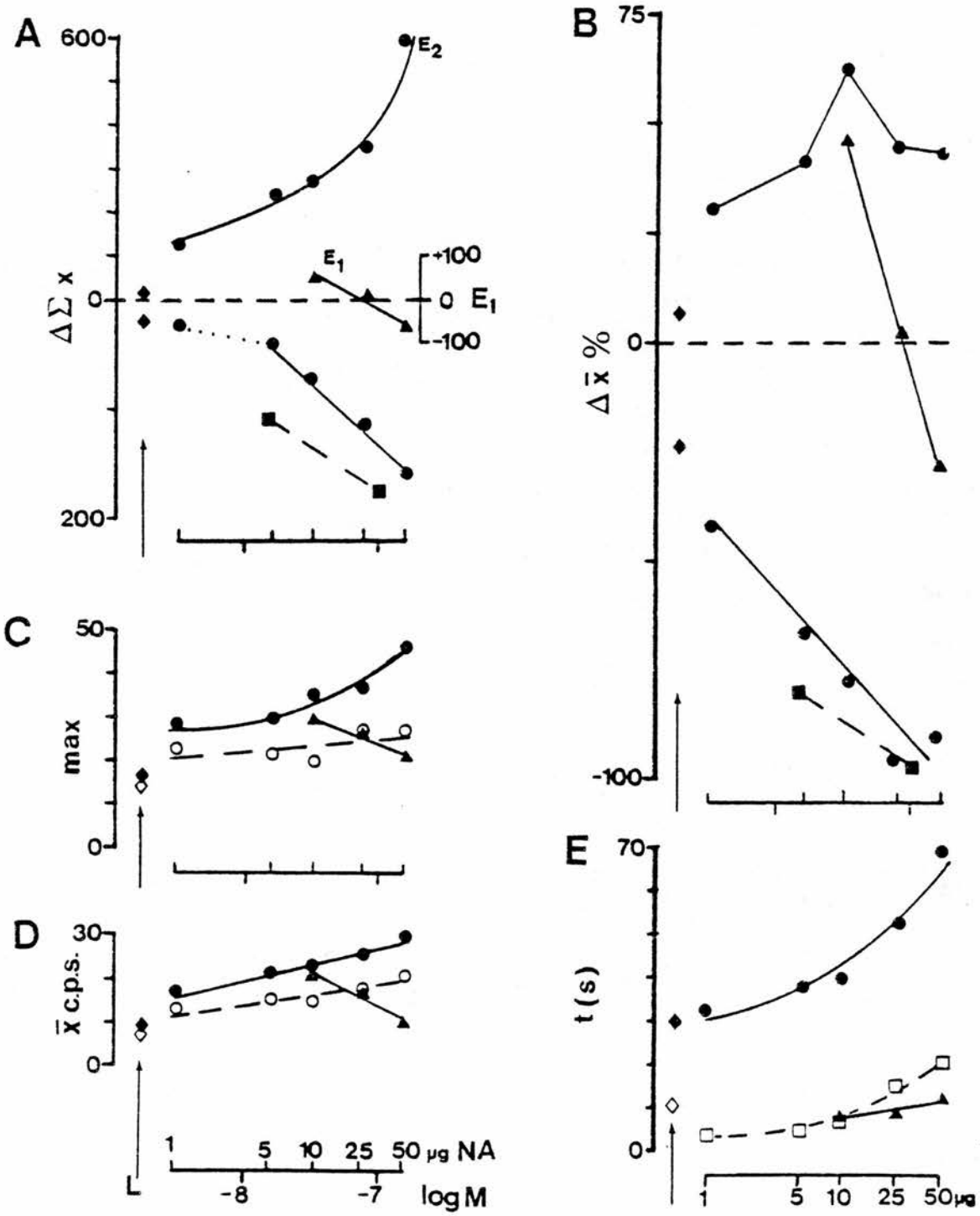
B: The same results expressed in terms of $\Delta\bar{x}\%$, related to dose.

C: Maximum discharge in the pre-injection control period (O), the period of NA-evoked E_2-excitation (●), and of E_1-excitation (▲), plotted against dose.

D: Mean discharge in the pre-injection control period (O), the period of NA-evoked E_2-excitation (●), and of E_1-excitation (▲), plotted against dose.

E: Duration of chemodepression (□), E_1-chemoexcitation (▲), and E_2-chemoexcitation (●) evoked by NA, plotted against dose of NA.

◇ or ◆ = effect of injecting Locke solution, 0.3 ml. Lines were fitted by eye, to give a better assimilation of the data points.


A
600
E2
E1
+100
0
E1
-100
ΔΣ x
0
200
B
75
Δ x̄ %
0
-100
C
50
max
0
D
30
x̄ c.p.s.
0
1
5
10
25
50 µg NA
-8
-7
log M
E
70
t (s)
0
1
5
10
25
50µg

the maxima attained in response to NA.

In general $\Delta\bar{x}\%$ appeared to be a poor index of the dose-dependency of chemoreceptor responses (e.g. Fig. 3.8A) since the change in mean discharge frequency, compared to pre-injection control frequency, was a near-constant function of dose. An obvious dose-dependency of the magnitude of the response emerged when discharge was integrated with respect to pre-injection control discharge ($\Delta\Sigma x$; Fig. 3.8A); this was related to dose in virtually all experiments. The integrated response takes account not only of changes in discharge frequency but also of the duration of the response, which was found to be a linear, or more often, an exponential function of dose (Fig. 3.8D). The 'excitability' of the chemoreceptors was not obviously related to dose in these experiments since the parameters Δmax (cf. Fig. 3.8B) and the drug-evoked increase in mean discharge ($\Delta\bar{x}$) were not dependent upon dose. These measures of chemoreceptor excitation usually showed a small but constant increase, with no apparent dependency upon dose of NA.

Where the two excitatory components could be studied separately the magnitude of the integrated response for E_2-excitation was greater than that of the E_1-type (Fig. 3.7), due to the considerably longer duration of the more delayed type of excitation (Fig. 3.7E).

With lower doses of NA E_1-type excitation appeared to be the major component of the overall excitatory response to NA, whilst E_2-effects predominated at higher doses (cf. Fig. 3.9).

After administration of DA-antagonists such as DP it has often been noticed that there is an increase in basal discharge frequency (e.g. McQueen, 1984). After DP NA-induced chemoexcitation was characterised by an increase in the excitability of the chemoreceptors, as shown by the emergence of a larger and clearly dose-dependent increase

Figure 3.8. Analysis of the response of 2-3 chemoreceptor units to intracarotid injection of NA.

A: $\Delta\Sigma x$ (●) and $\Delta\bar{x}\%$ (O) for the chemodepression and chemoexcitation evoked by NA, plotted against dose. For both depression and excitation $\Delta\Sigma x$ increases (positively and negatively respectively) as dose is increased, whilst $\Delta\bar{x}\%$ remains largely a constant function of dose.

B: Maximum number of action potentials counted in any one second of the control period (O) and of the NA-evoked excitation (●), plotted against dose.

C: Mean discharge in the pre-injection control period (O), and in the period of increased discharge following injection of NA (●).

D: Duration of the chemodepression evoked by NA (□), and chemoexcitation (●) which follows NA-evoked chemodepression, plotted against dose.

◇ or ◆ = effects of injecting Locke solution, 0.3 ml. Lines were fitted by eye, to give a better assimilation of the data points.

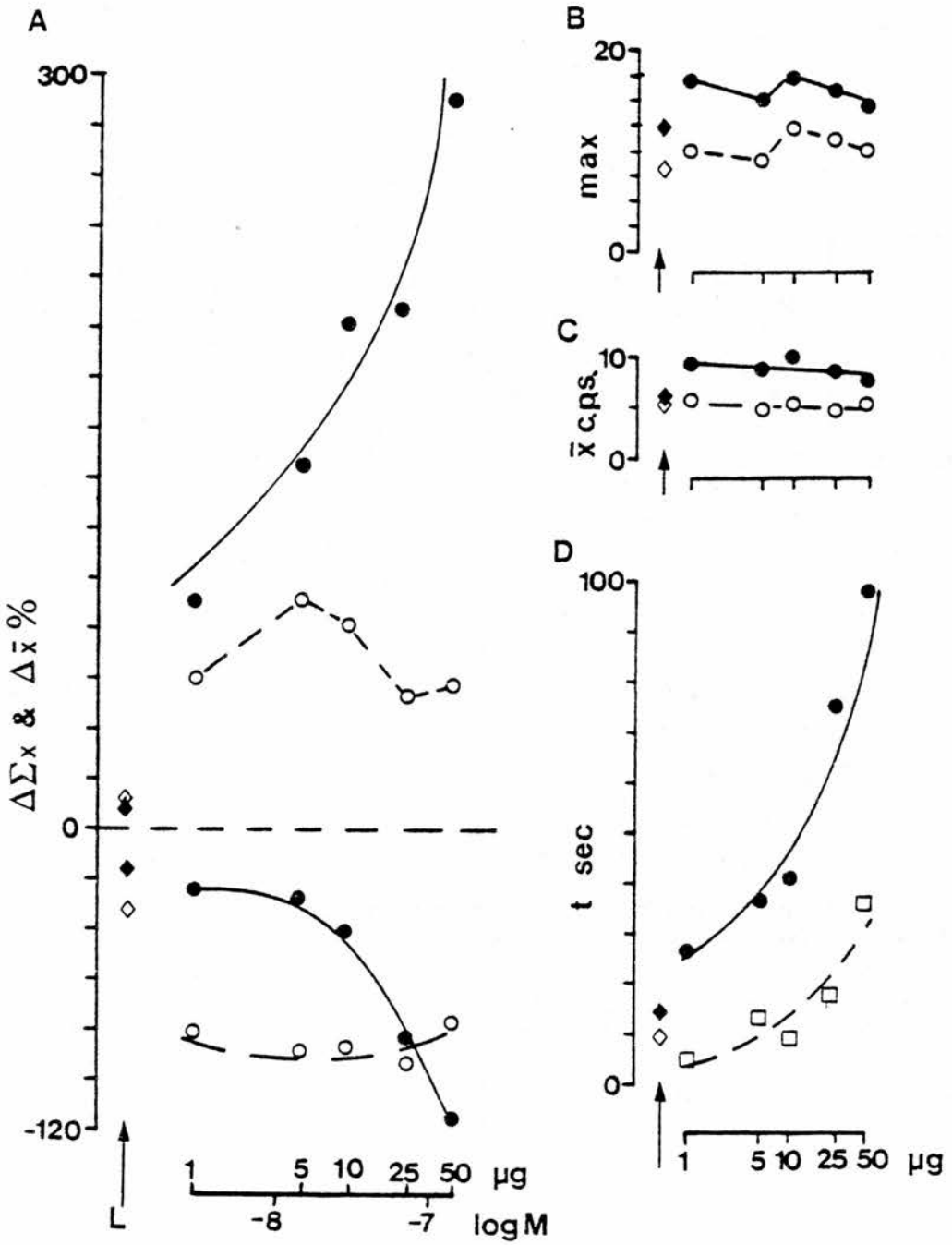

A
300
0
-120
ΔΣx & Δx̄ %
L
1
5
10
25
50
µg
-8
-7
log M
B
20
max
0
C
10
x̄ c.p.s.
0
D
100
t sec
0
1
5 10 25 50 µg

Figure 3.9. Responses of a single chemoreceptor unit to the i.c. injection of NA.

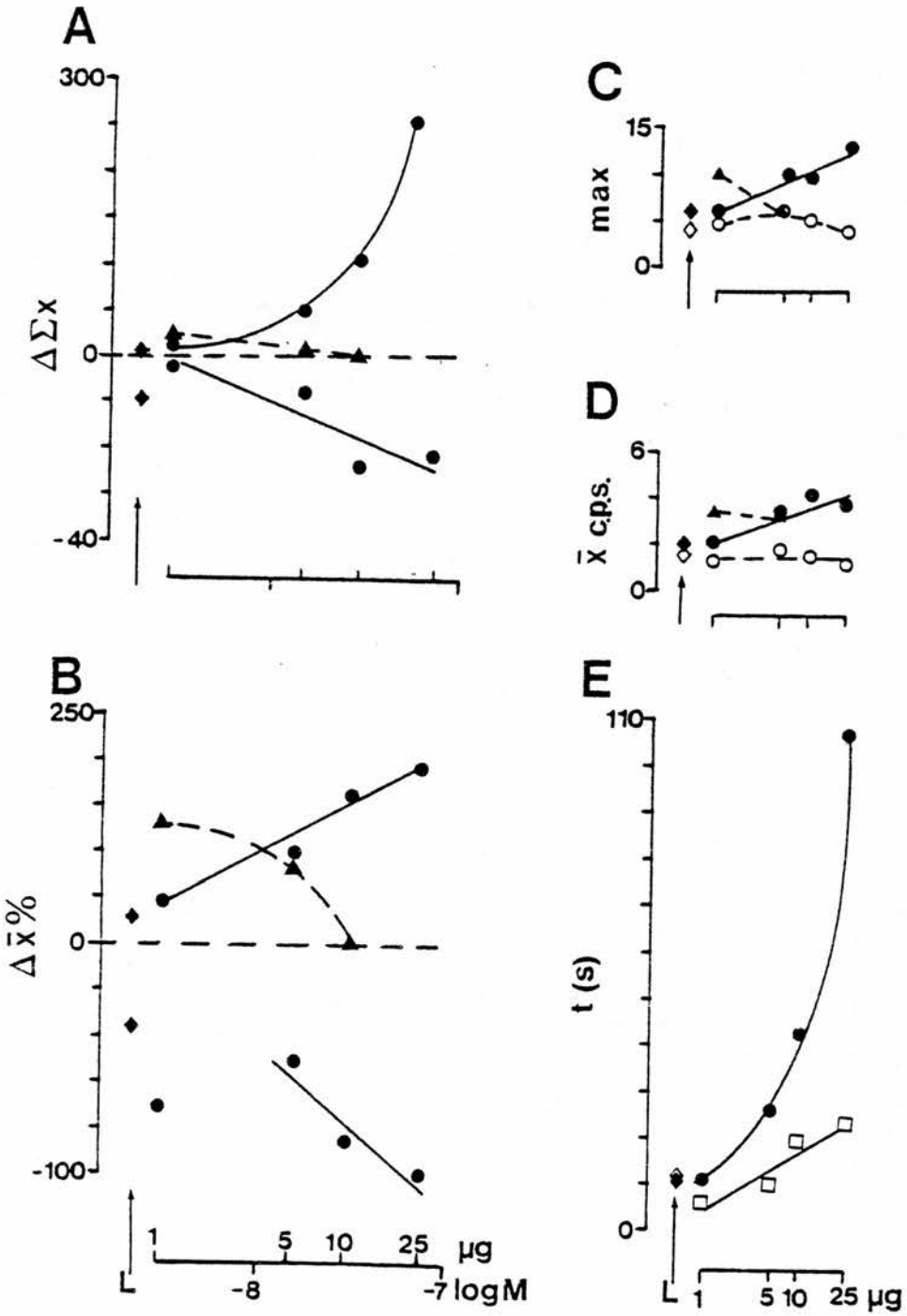
A: Integrated chemodepression and E_2-excitation evoked by NA (●—●) plotted axgainst dose. Also included is the E_1-type chemoexcitation evoked by 1, 5, and 10 µg of NA (▲ - ▲).

B: $\Delta\bar{x}\%$ for NA-evoked chemodepression, E_2-chemoexcitation (●), and E_1-excitation (▲), expressed as a percentage change from pre-injection control discharge, and plotted against dose.

C: Maximum discharge (in any one second), and D, mean discharge frequency (per second) for pre-injection control (O), E_2-excitation (●), and E_1-excitation (▲) plotted against dose.

E: Duration of the chemodepression (□) and the overall (gross) excitation (●) evoked by NA, plotted against dose.

◊ or ◆ = effect of injecting Locke solution, 0.3 ml. Lines were fitted by eye to give a better assimilation of the data points.


A
300
0
-40
ΔΣx
C
15
0
max
D
6
0
x̄ c.p.s.
B
250
0
-100
Δx̄%
1
5
10
25
µg
-8
-7 logM
E
110
0
t (s)
1
5 10
25 µg

in Δmax and $\Delta\bar{x}$ (Figs 3.10, 3.16).

3.2.5 Chemoexcitatory responses to selective adrenoceptor agonists and the effects of selective antagonists.

Since NA is a non-selective agonist with potential to activate α_1-, α_2-, or β_1- receptors, the capacity of putatively more selective adrenoceptor agonists to cause excitation of the chemoreceptors was examined to determine the possible contribution to the NA-evoked response of effects mediated by particular receptor subtypes.

3.2.6 Effects of α-selective agonists and antagonists.

3.2.6.1 Phenylephrine (α_1-agonist) and corynanthine (α_1-antagonist).

In nine cats PHEN(1 μg - 2 mg i.c.) caused only slight changes in chemoreceptor discharge, including variable but insignificant chemoexcitation (following depression, if present - see Fig. 3.12), which did not appear to increase with dose. Although this feeble excitatory effect was commonly, though not always, inversely related to dose (cf. Fig. 3.12) it was not comparable to E_1-excitation evoked by NA (see above), being neither intense nor transient. The maximal discharge (Δmax) evoked by PHEN was much smaller than that following injection of NA. Delayed dose-related chemoexcitation concomitant with the marked hypertensive increase in systolic and diastolic blood pressure was not observed in any experiment, although the pressor responses to PHEN (see later) were as great as those elicited by similar doses of NA.

The α_1-selective antagonist COR (0.1-1 mg kg^{-1} i.c.) was injected in four cats; small chemoexcitatory responses to NA injection (in part-

Figure 3.10. Effects of DP (0.1 mg kg^{-1}) and MET (2.9 mg kg^{-1}) upon responses of a single chemoreceptor unit to i.c. injection of NA.

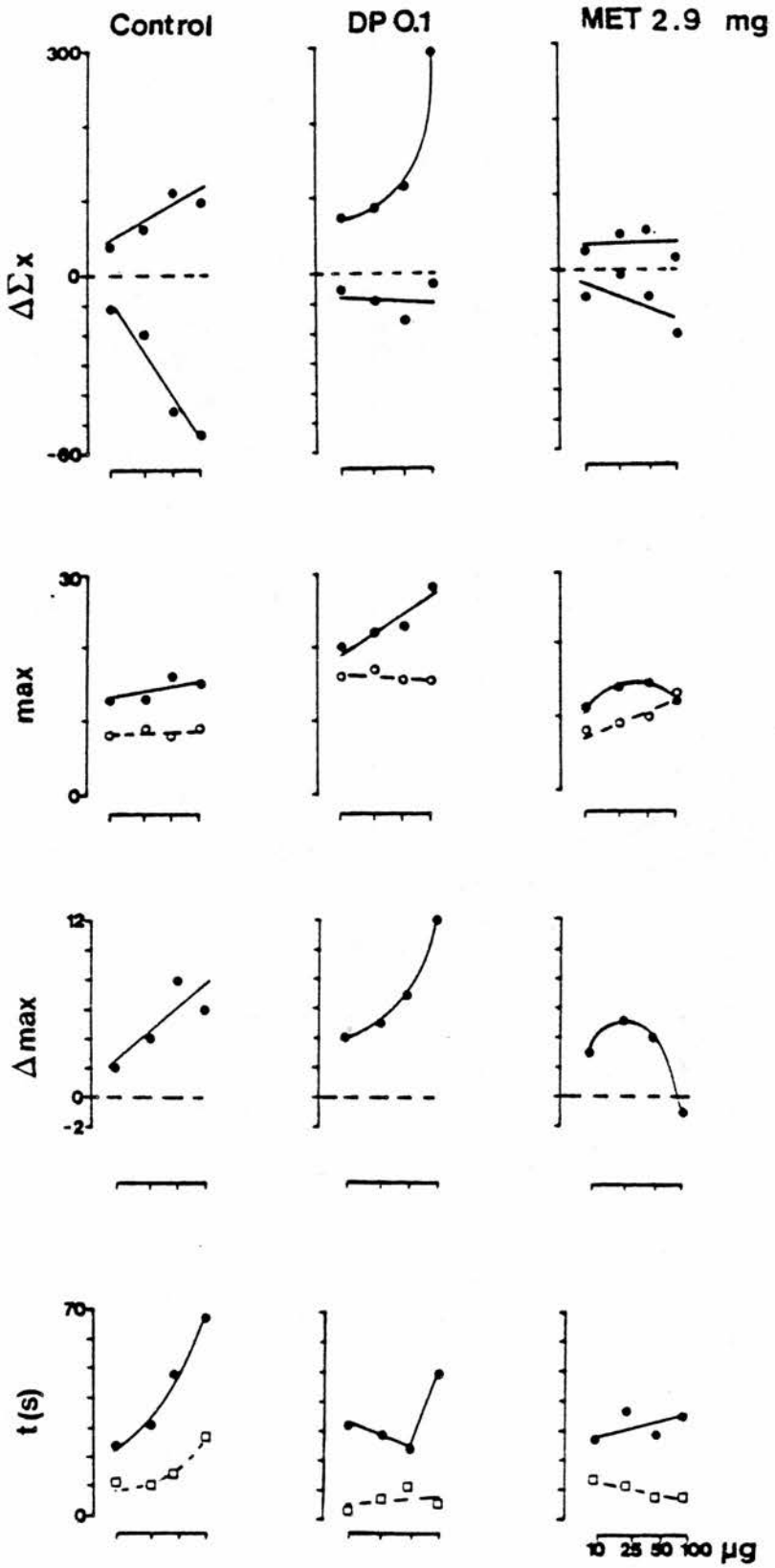
Top panels: Chemodepression and chemoexcitation quantified in terms of $\Delta\Sigma x$, and plotted against dose. Chemodepression was reduced by DP; chemoexcitation was enhanced by DP, and reduced by MET.

Second row: Maxima (per second) for control (O) and NA-evoked chemoexcitation (●). Before antagonists, excitation in response to NA was largely unrelated to, or a near-constant function of dose. After DP background discharge increased, as evidenced by the increase in maximum discharge, and the maximum for NA-evoked excitation became more dose-dependent. Both maxima were reduced by MET.

Third row: Δmax plotted as a function of dose.

Bottom panels: Duration of the NA-evoked chemodepression (□), and chemoexcitation (●). The duration of both phases of the response to NA were generally reduced, and less dose-dependent following DP, with no appreciable further changes observed after injecting MET.

Lines were fitted by eye to give a better assimilation of the data points.


Control
DP 0.1
MET 2.9 mg
ΔΣx
300
0
-60
max
30
0
Δmax
12
0
-2
t(s)
70
0
10 25 50 100 µg

icular E_1-type) still occurred after this antagonist (cf. Figs 3.16, 3.25), and biological activity of the drug was gauged from its antagonism of PHEN-induced hypertension and its partial blockade of the pressor effects of NA (see later).

3.2.6.2 Oxymetazoline (α_2-agonist) and rauwolscine (α_2-antagonist).

OXM (0.1-200 µg i.c.) was injected in five cats. Chemoexcitation was evoked by injections of OXM in three experiments, the threshold dose being in the order of 10 µg (Fig. 3.11), but, in common with NA, quantification of the response was highly varied when different experiments were compared. As with NA there was some evidence in the one experiment where tested (Fig. 3.11) of potentiation of chemoexcitation evoked by this agonist, after injecting BET (see below).

The antagonist RAU (0.01-4 mg kg^{-1}) was injected in ten experiments; chemoexcitation in response to NA injection was still apparent in five out of six (83%) of the recordings where it was possible to examine the effects of NA after the antagonist (e.g. Fig. 3.25), but the excitatory effects of OXM were reduced or abolished (Fig. 3.11).

3.2.7 Chemoexcitatory responses to β-adrenoceptor agonists and the effects of selective β-adrenoceptor antagonists.

3.2.7.1 Effects of isoprenaline (β_1/β_2 agonist).

Injection of ISO in seven cats caused a general increase in chemoreceptor discharge, without any initial depression. The effect was neither rapid nor intense, unlike the early (E_1) excitation frequently evoked by NA, but was comparable to the prolonged E_2-type excitation

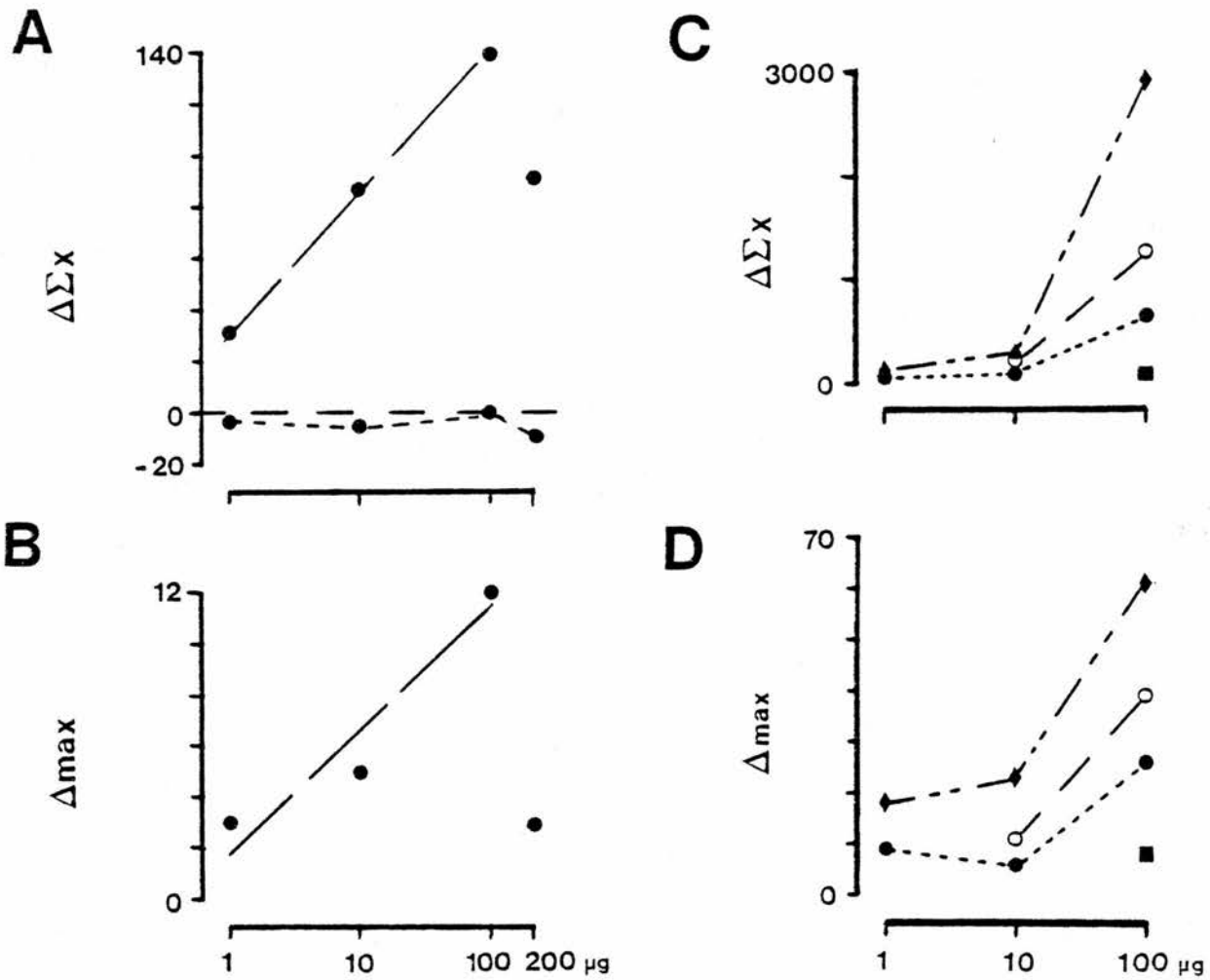


Figure 3.11. Effects upon chemoreceptor discharge of injecting OXM, 1-200 µg i.c.; the major component of the response was chemoexcitation, which followed a small, non-dose-dependent depression of discharge.

A. Responses (from a single experiment) integrated with respect to pre-injection control discharge.

B. Δmax for the excitatory component of the response.

Qualitatively similar excitatory responses were obtained in a separate experiment, although there was no initial depression of discharge:

C. Responses integrated with respect to pre-injection control discharge, before (O) and after BET 100 µg kg^{-1} (◆), RAU 100 µg kg^{-1} (●) and RAU 1 mg kg^{-1} (■).

D. Δmax for the excitatory response, showing a potentiation after BET, and attenuation after administration of RAU.

seen in response to NA. The onset and duration of ISO-evoked excitation was concomitant with dose-dependent systemic hypotension (see later), and could persist for several minutes, according to dose. In two experiments dose-response data were obtained over a range of 0.001-100 µg, and chemoexcitation occured with doses as low as 0.1 µg. In both experiments $\Delta\Sigma x$ was dose-related although the correlation was poor in one. The parameter Δmax was an approximately linear function of dose, in contrast to changes in Δmax seen after NA (Fig. 3.12). After ISO injections basal discharge appeared to be 'reset' at a slightly higher frequency than in the pre-injection control, even though blood pressure returned to near control levels.

3.2.7.2 Effects of β_1-selective agonists.

The DA-derivative DOB was studied in three experiments, and the chemodepressant effects of this drug have been described above. No chemoexcitation occured in response to injecting 1-100 µg (i.c.), nor, in one experiment, to 5 mg of the drug injected intravenously.

The effects of PREN (β_1) were studied in three cats over the range 0.1 µg - 1 mg i.c., or, (n=1) 5 mg i.v. Dose response data were obtained in one experiment where integration of the responses showed a small excitatory effect, largely unrelated to dose (Fig. 3.13), following chemodepression. The parameter Δmax was log-linearly related to dose, even though the effect was small. Injection of 5 mg of the drug intravenously caused no obvious significant change in chemoreceptor discharge (Fig. 3.13).

3.2.7.3 Effects of the β_1-antagonist metoprolol.

The effects of MET upon chemoreceptor responses to NA were

Figure 3.12. A. Integrated responses of chemoreceptors, in the same experiment, to i.c. injection of PHEN (O—O), ISO (■ - - ■) and SAL (● —— ●) plotted on the same log-molar scale. Dose-related chemodepression for the response to PHEN was greater than that seen in response to β-agonists, which may be considered to be artefactual. ISO was more potent in stimulating excitatory responses than was SAL, and excitation evoked by PHEN was weak, and inversely related to dose. ◆ denotes effects of injecting Locke solution, 0.3 ml; lines were fitted to the data by the method of least squares.

B. Δmax for excitatory responses to PHEN (O—O), ISO (■ - - ■) and SAL (● — ●) plotted on the same log-molar scale. Data points in parentheses were omitted when calculating coordinates of the straight lines by the method of least squares.

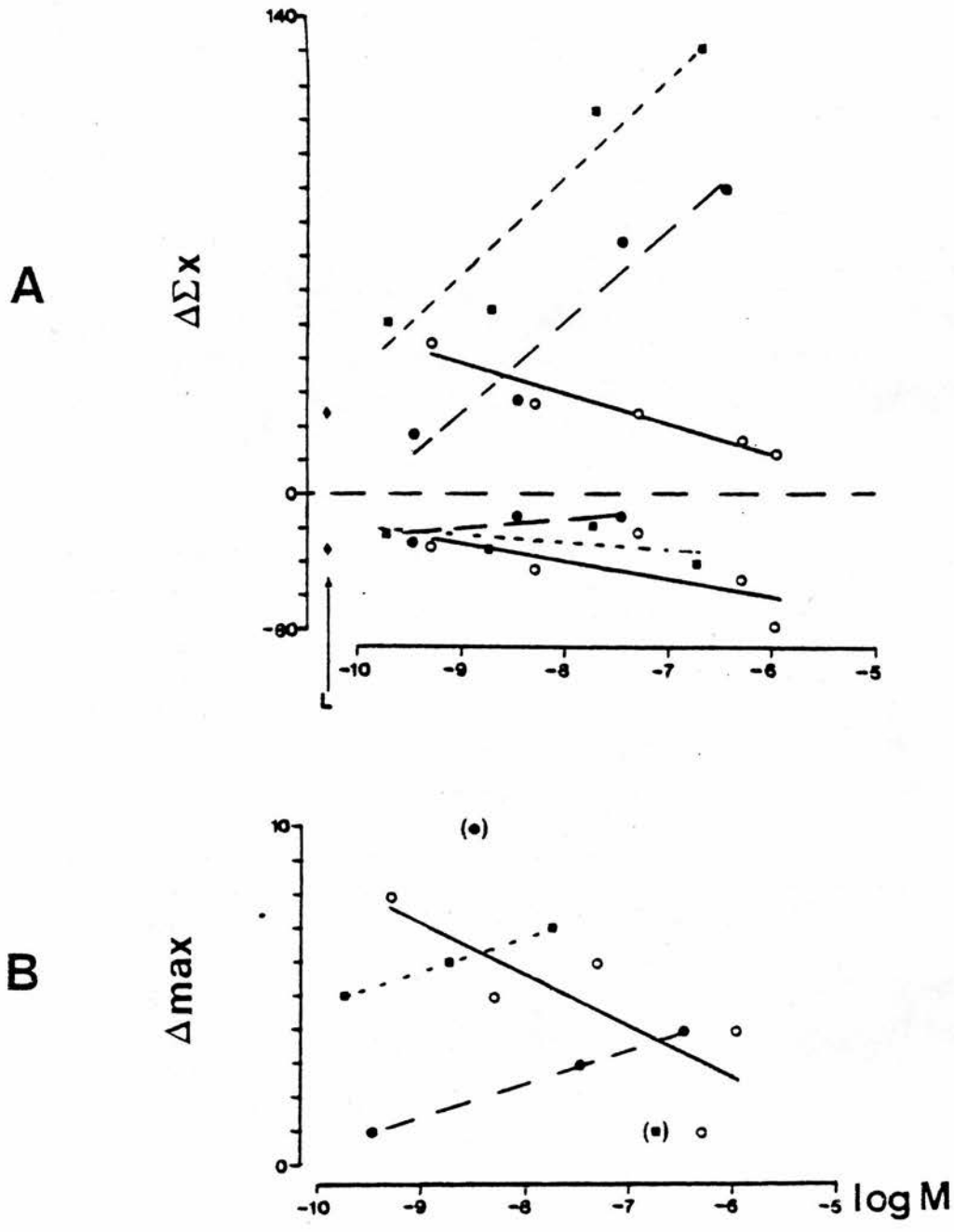
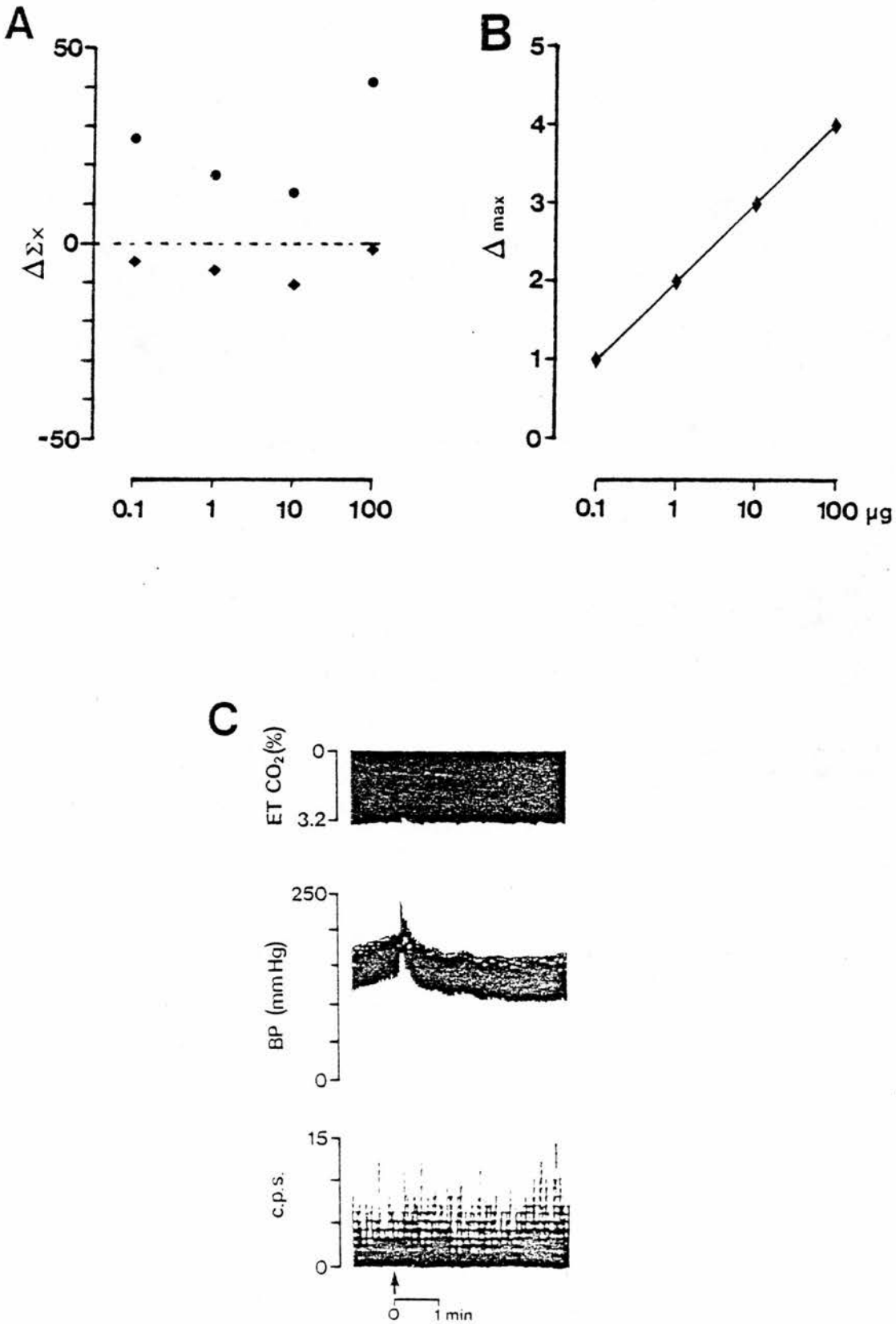

A
ΔΣx
140
0
-80
-10
-9
-8
-7
-6
-5
B
Δmax
10
0
(•)
(■)
log M

Figure 3.13. Response of chemoreceptors to injection of PREN (0.1-100 µg i.c.).

A. Integrated response ($\Delta\Sigma x$) for initial chemodepression and subsequent chemoexcitation plotted against dose.

B. Δmax during excitation plotted against dose. The line was fitted to the data by the method of least squares.

C. Effects upon end tidal CO_2 (ET CO_2, calibration 0-3.2%) femoral arterial blood pressure (BP, mm Hg) and chemoreceptor discharge (c.p.s. - analogue signal proportional to rate of discharge) of injection of PREN, 5 mg i.v. Although blood pressure was increased transiently, during and immediately after the injection, chemoreceptor discharge was not obviously affected.


A
50
0
-50
ΔΣx
0.1
1
10
100
B
5
4
3
2
1
0
Δ max
0.1
1
10
100 µg
C
ET CO2(%)
0
3.2
250
BP (mmHg)
0
15
c.p.s.
0
1 min

studied in nine experiments. The range of doses used was 0.1 μg - 1 mg, and this was extended to a maximum dose of 3 mg kg^{-1} in one experiment.

Although the rate of chemoreceptor discharge usually decreased after injection of the antagonist, quantitative analysis of the altered chemoexcitatory response to NA was not comparable between experiments. When expressed in terms of $\Delta\Sigma x$, NA-induced chemoexcitation was decreased after MET. The threshold dose of antagonist after which the excitatory response appeared to be reduced varied between experiments. MET was effective at doses of 1 μg kg^{-1} (n=1), 100 μg kg^{-1} (n=1), or 1 mg kg^{-1} (n=2) (see Fig. 3.14). This reduction of $\Delta\Sigma x$ was generally associated with a decrease in Δmax and $\Delta\bar{x}$ (Fig. 3.14), but with variable changes in the duration of the excitatory phase.

Low doses of MET (1-10 μg kg^{-1}) were frequently followed by an enhanced chemoexcitatory response to NA both in the absence of DP (Fig. 3.14) or following prior administration of DP. This was coincident with an elevation of resting heart rate (accompanied by a decreased chronotropic response to injected NA - see later) following low doses of MET.

In experiments where E_1- and E_2- type excitatory responses to NA could be studied separately E_1-excitation appeared to be largely unaffected by MET, whilst the later E_2-excitation was more susceptible to blockade by this antagonist. E_1-excitation appeared to persist after MET, even when chemodepression was more marked (Fig. 3.14); under such circumstances quantitative analysis of this 'excitation' with respect to the pre-injection control may result in negative values of $\Delta\Sigma x$, Δmax, or $\Delta\bar{x}$, even when it is clear that the chemoreceptors are being excited, albeit transiently (Fig. 3.15).

Figure 3.14. Responses of a single chemoreceptor unit to NA (1-100 µg i.c.), before (A) and after (B-E) injection of MET (0.1-3 mg kg^{-1} i.c.).

Top panels: Chemoreceptor discharge during chemodepression and chemoexcitation integrated with respect to pre-injection control discharge ($\Delta\Sigma x$), and plotted against dose. Chemodepression was enhanced following higher doses of the antagonist whilst excitation was reduced.

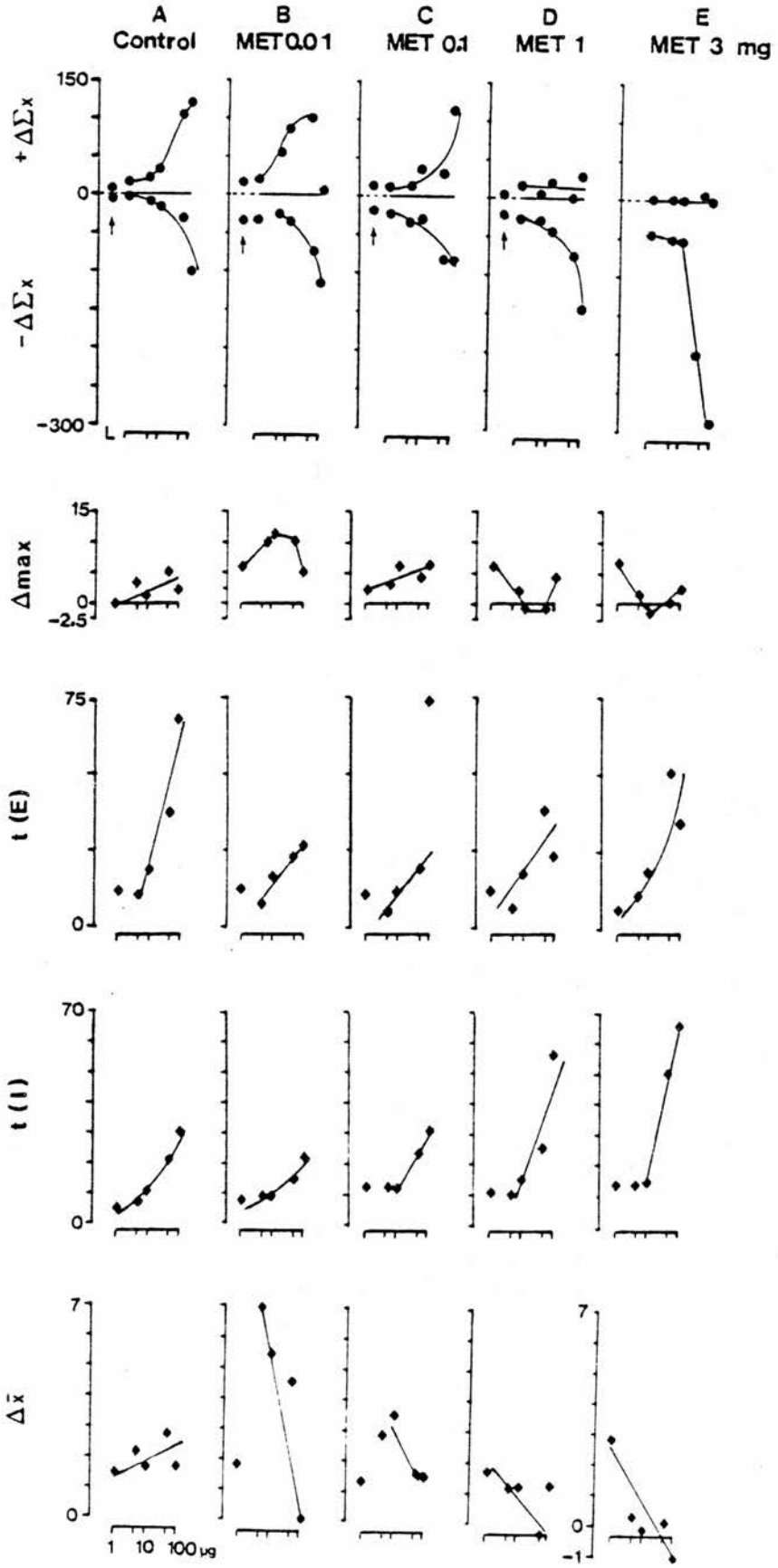
Second row: Δmax for the chemoexcitation evoked by NA. This parameter was increased after a low dose of antagonist, but reduced (at mid-range doses of NA) following higher doses of MET.

Third row: Duration, in seconds, of the chemoexcitatory response.

Fourth row: Duration, in seconds, of the chemodepressor response, showing a general tendency to increase with successive doses of antagonist.

Bottom panels: $\Delta\bar{x}$ for NA-evoked chemoexcitation, which was increased after a low dose of the antagonist, and reduced by higher doses.

Arrows indicate the effect of injecting Locke solution, 0.3 ml. Lines were fitted to the data by eye.


A
Control
B
MET0.01
C
MET 0.1
D
MET 1
E
MET 3 mg
+ΔΣx
-ΔΣx
150
0
-300
L
Δmax
15
0
-2.5
t (E)
75
0
t (I)
70
0
Δx̄
7
0
1 10 100 µg
7
0
-1

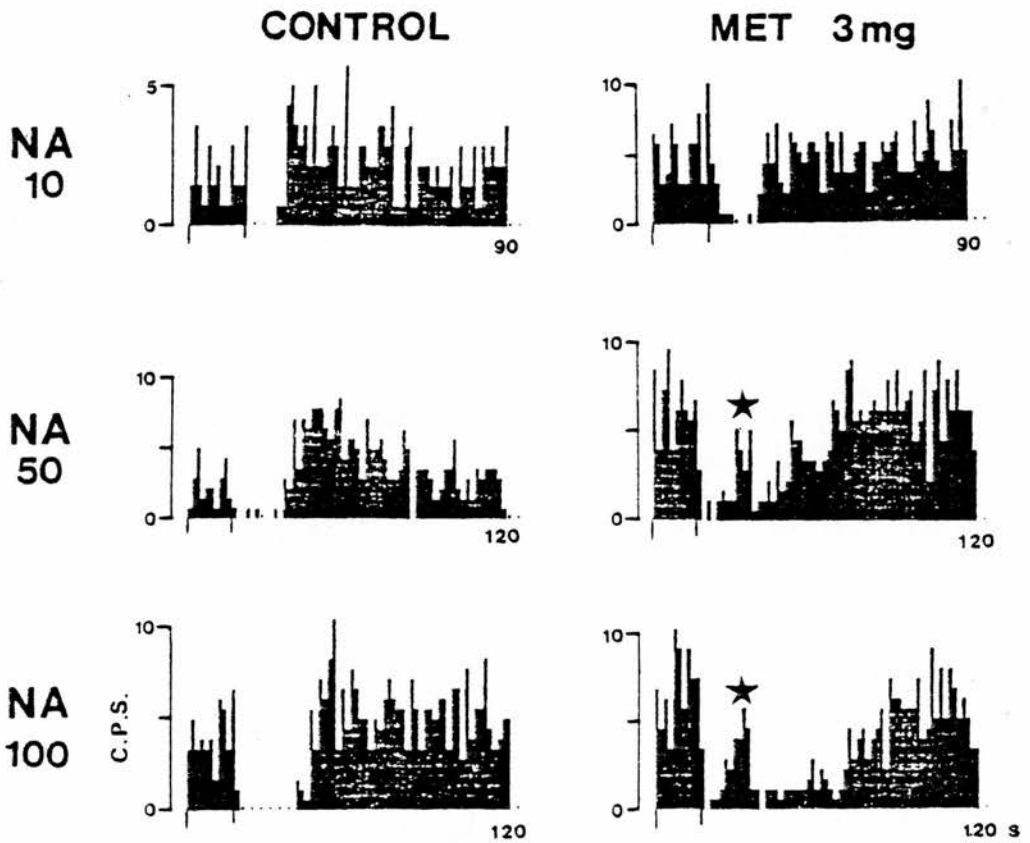


Figure 3.15. Computer-drawn plots of chemoreceptor discharge (single unit recording) as a function of time. Responses to i.c. injections of NA 10, 50, and 100 µg are shown before and after MET 3 mg kg^{-1}. NA-evoked chemodepression was greatly prolonged after the antagonist, although depression of discharge was less absolute than in the control. Asterisks denote a presumptive 'E_1'-excitation occuring during the period of reduced chemoreceptor discharge. Data were collected over 90 or 120 s, as indicated, and the pre-injection control period is delineated by the vertical lines below each plot.

'Reversal' of the MET-blockade of NA-induced chemoexcitation (i.e. prominent chemoexcitation occured once again in response to injection of NA) was noted following injection of ICI 118551 (n=3; cf. Fig. 3.16) or RAU (1 mg kg^{-1} - n=1).

3.2.7.4 Effects of the β_1-antagonist betaxolol.

The chemoreceptor response to NA was studied after BET in seven experiments. The effects upon basal chemoreceptor discharge were variable, with either a slight increase or decrease, or no significant change in frequency being noted.

In the absence of other antagonists (n=4) the administration of BET 1-10 or even 100 µg kg^{-1} was followed by an enhanced excitatory response to injected NA. Where they could be separately identified both E_1- and E_2- excitatory components of the response to injected NA were potentiated after these lower doses of antagonist (Fig. 3.17), Δmax being most obviously increased. Raising the dose of BET to 1 mg kg^{-1} or more resulted in the attenuation or abolition of E_2-excitation, whereas E_1-excitation persisted as a near constant function of dose (Fig. 3.17), and continued to be a characteristically 'intense' effect (cf. Fig. 3.18).

Two features of this persistent chemoexcitation are worthy of note. First, it occured during the period of enhanced NA-evoked chemodepression which followed high doses of BET. Second, the effect was either an intense and transient excitation, when discharge frequency clearly exceeded that of the pre-injection control period, or it was a small, but none-the-less identifiable entity (cf. the similar response after MET, Fig. 3.15), and although discharge frequency was not greater than in the control period, measurements of Δmax or $\Delta\bar{x}$

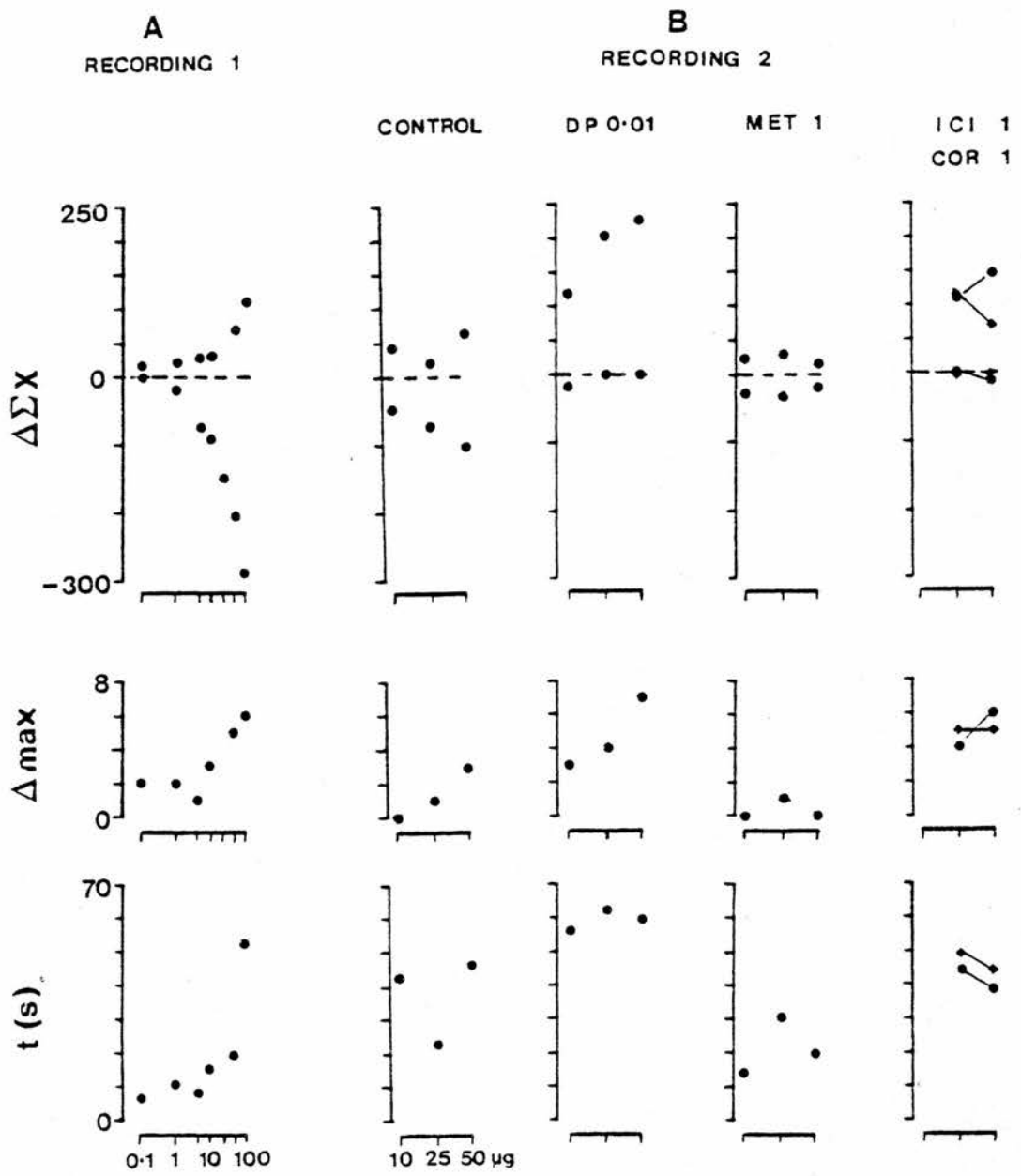


Figure 3.16. A and B: Responses of two different recordings , in the same animal, to i.c. injection of NA; in the second recording, results were obtained also after DP (10 µg kg^{-1}), MET (1 mg kg^{-1}), ICI 118551 (●; 1 mg kg^{-1}) and, finally, COR (◆; 1 mg kg^{-1} - plotted on the same axes as the results after ICI 118551). Data are presented as integrated responses ($\Delta\Sigma x$), Δmax, and the duration of the excitatory effect.

Figure 3.17. Excitatory responses to NA in an experiment where both types of excitation could be analysed separately. Early E_1-(open circles) and delayed E_2-(closed circles) components are plotted against dose; data are presented for the changes in (A) Δmax, (B) $\Delta\bar{x}$, (C) $\Delta\Sigma x$, and (D) duration of the response.

Duration of E_1-excitation was a near-constant function of dose, unlike that of E_2-excitation. The excitability of the system (e.g. Δmax), contributing to the magnitude of $\Delta\Sigma x$ was decreased by increasing doses of the antagonist BET (0.1-5 mg kg^{-1}), with respect to E_2-excitation, whilst E_1-excitation remained largely unnaffected.

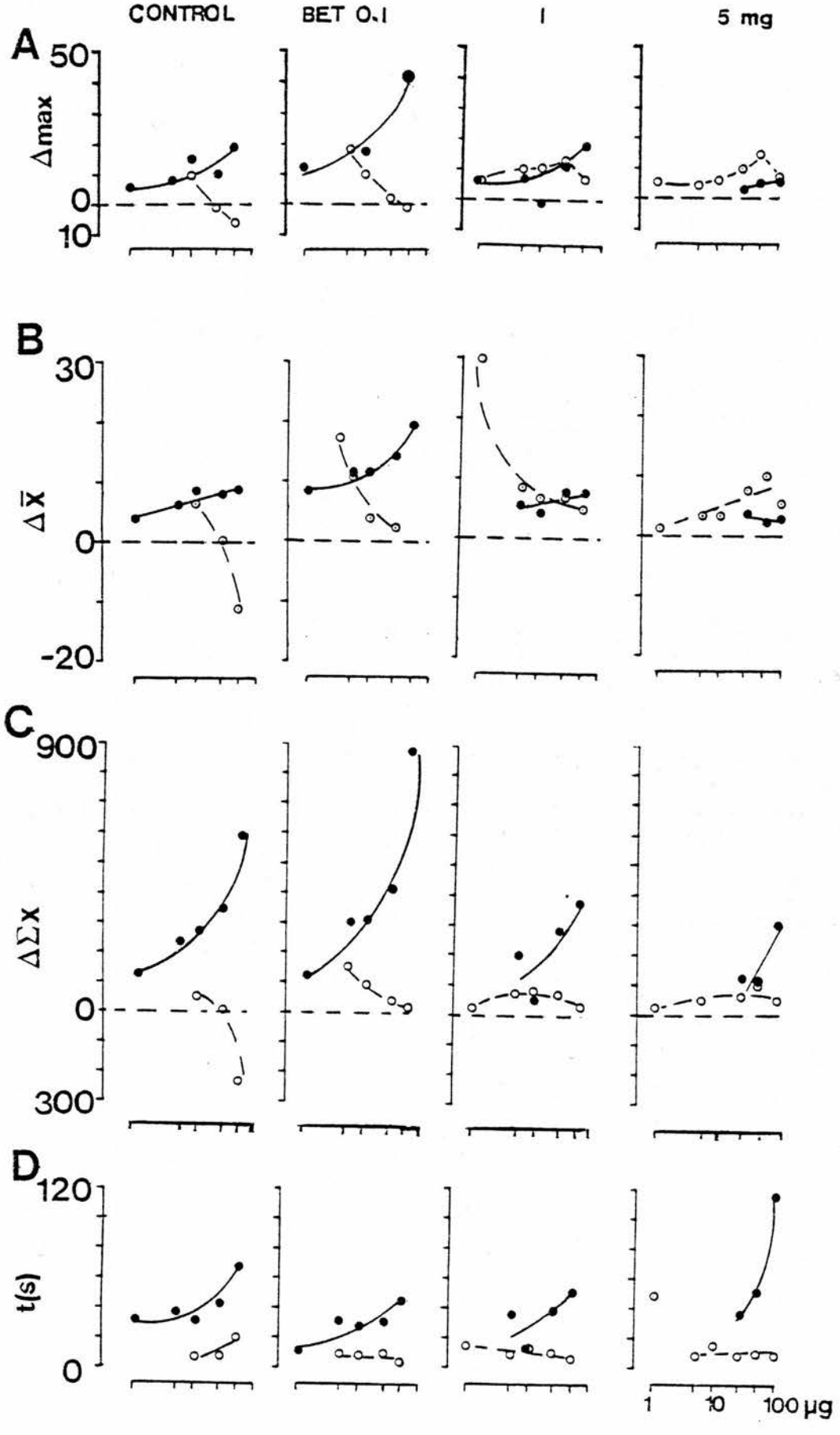
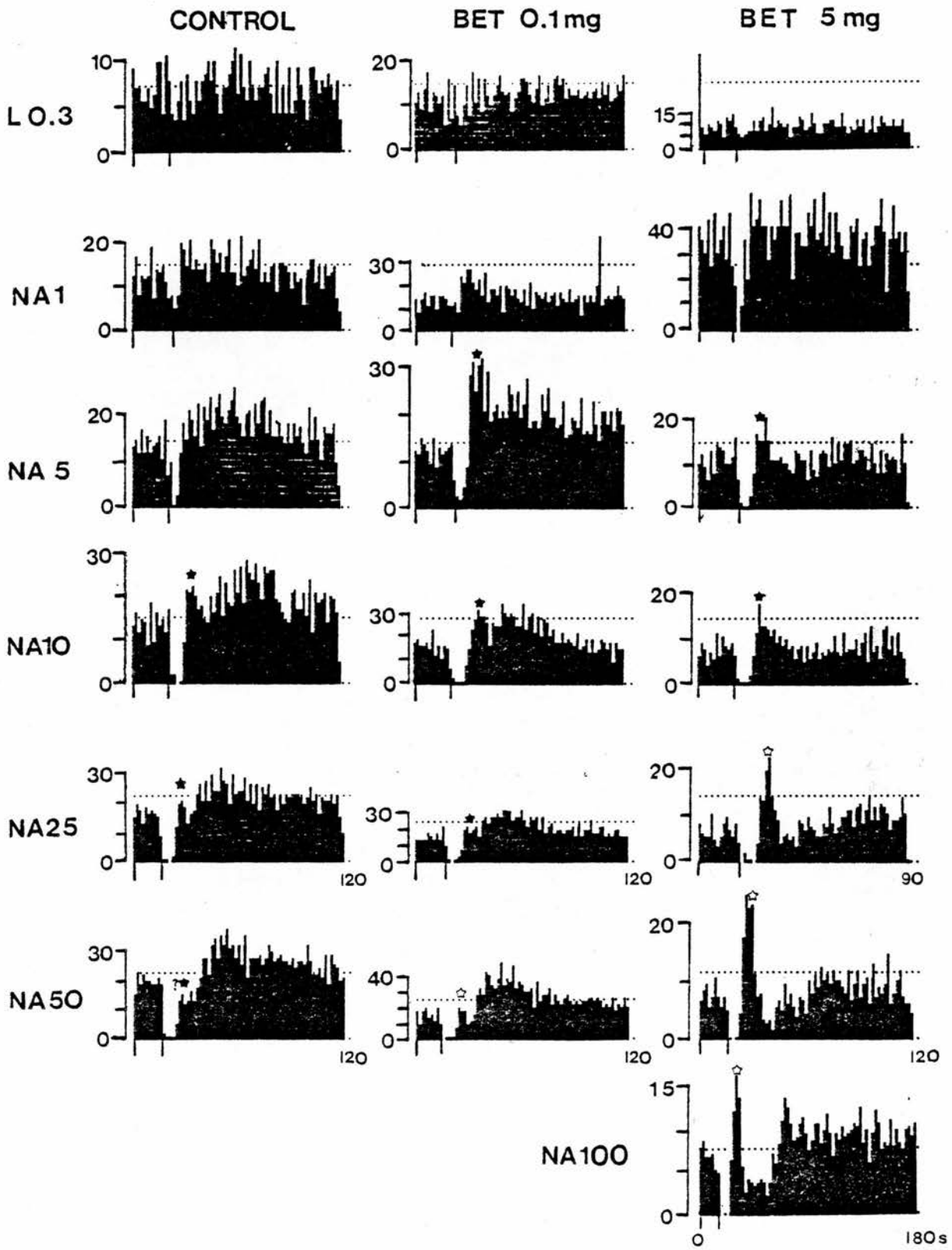

CONTROL
BET 0.1
1
5 mg
A
50
Δmax
0
10
B
30
Δx̄
0
-20
C
900
ΔΣx
0
300
D
120
t(s)
0
1
10
100 µg

Figure 3.18. Computer-drawn plots of chemoreceptor discharge as a function of time. Responses to injection of Locke solution, 0.3 ml, and NA 1, 5, 10, 25, 50, and 100 µg i.c. are shown, before and after BET 0.1 and 5 mg kg^{-1}, in the same experiment. A solid asterisk denotes E_1-excitation, and an unfilled asterisk denotes an E_1-excitation occurring during the period of chemodepression, and for which small or negative values of parameters such as Δmax may be obtained (cf. NA 50 µg , after BET 0.1 mg kg^{-1}) when comparing with the pre-injection control. The paired vertical lines beneath each illustration delineate the pre-injection control period.

Calibration of the ordinate in each case is in counts per second (c.p.s.). Data collection was for 90 s, or 120-180 s, as indicated.

(continued)


CONTROL
BET 0.1mg
BET 5 mg
L 0.3
NA 1
NA 5
NA 10
NA 25
NA 50
NA 100
10
0
20
15
40
30
120
90
180s

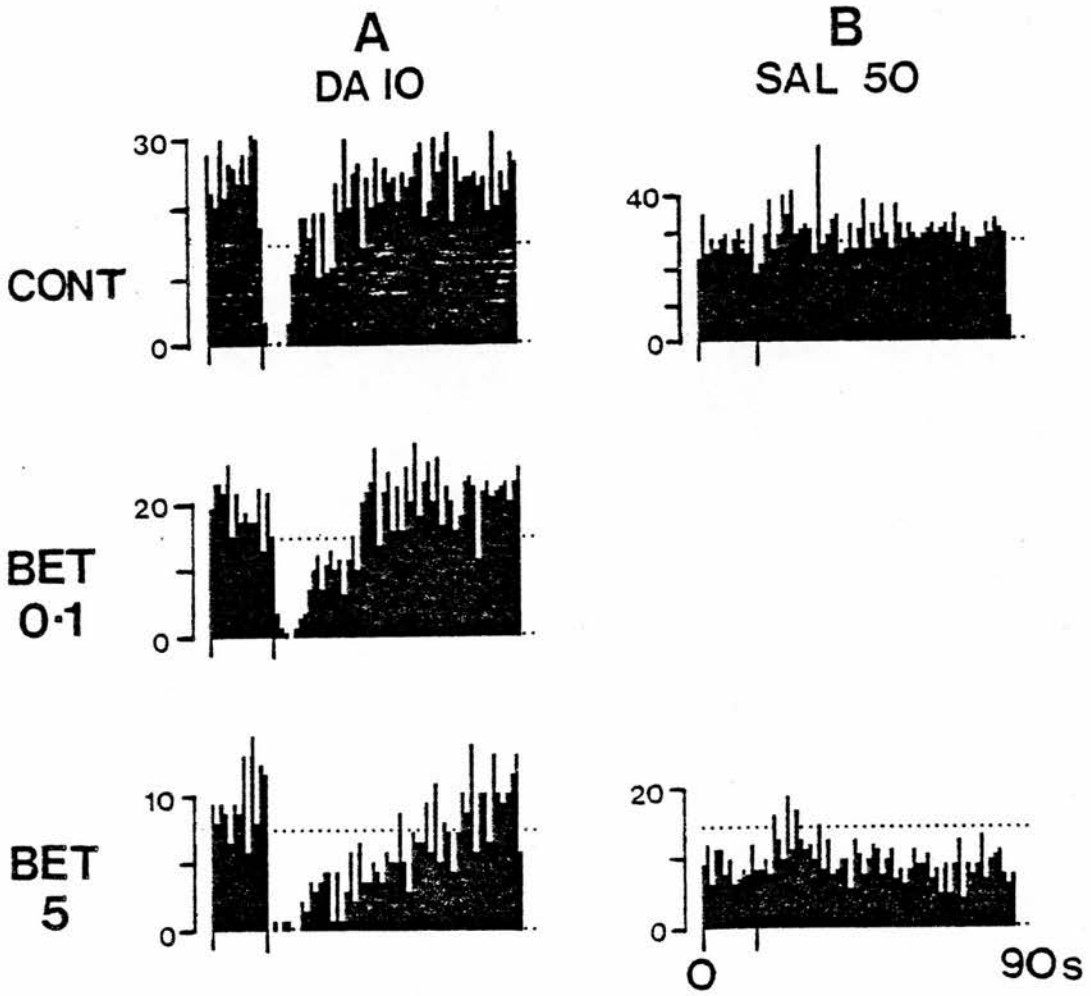


Figure 3.18. Computer-drawn plots of chemoreceptor discharge as a function of time, from the same experiment as shown previously. Responses to injection of (A) DA 10 µg and (B) SAL 50 µg, before (CONT) and after BET 0.1 and 5 mg kg^{-1}. The paired vertical lines beneath each illustration delineate the pre-injection control period.

Calibration of the ordinate in each case is in counts per second (c.p.s.). Data collection was for 90 s.

were linearly related to dose (though negative in sign, with respect to control).

3.2.7.5 Effects of salbutamol (β_2-agonist) and ICI 118551 (β_2-antagonist).

Single injections or the injection of a range of doses of SAL (0.1 µg - 1 mg i.c.) in thirteen cats caused chemoexcitation. Eight dose-response curves for the SAL-evoked chemoexcitation were obtained (Fig. 3.19), and there was a linear relationship between $\Delta\Sigma x$ and dose in seven (88%). There was, however, considerable variation in the magnitude of the response between experiments. Mean discharge during chemoexcitation, expressed as a percentage change from pre-injection control, did not show such a clear dependence upon dose (cf. Fig. 3.20 [2]). The disparity between pre-injection control and post-injection max discharge frequencies (Δmax - Fig. 3.20 [3]) did not obviously increase with dose (but see Fig. 3.22). When the mean discharge frequency of the pre-injection and SAL-evoked chemoexcitation were plotted on the same axes (Fig. 3.20 [5]), it can be seen that though there was a tendency for background discharge to increase the change in mean frequency evoked by SAL was nearly constant; thus $\Delta\Sigma x$ was predominantly a function of the duration of the response (Fig. 3.20 [4]) rather than of mean frequency, as was also observed with NA.

Chemoexcitation evoked by SAL was not consistently altered following injection of the β_2-antagonist ICI 118551 (0.1 or 1 mg kg^{-1} i.c. or i.v.). ICI 118551 substantially reduced SAL-evoked chemoexcitation in one experiment only (Fig. 3.21 A,B), and in two other experiments caused no obvious change (e.g. Fig. 3.22), or a slight potentiation of the effect. The β_1-selective antagonists BET or MET

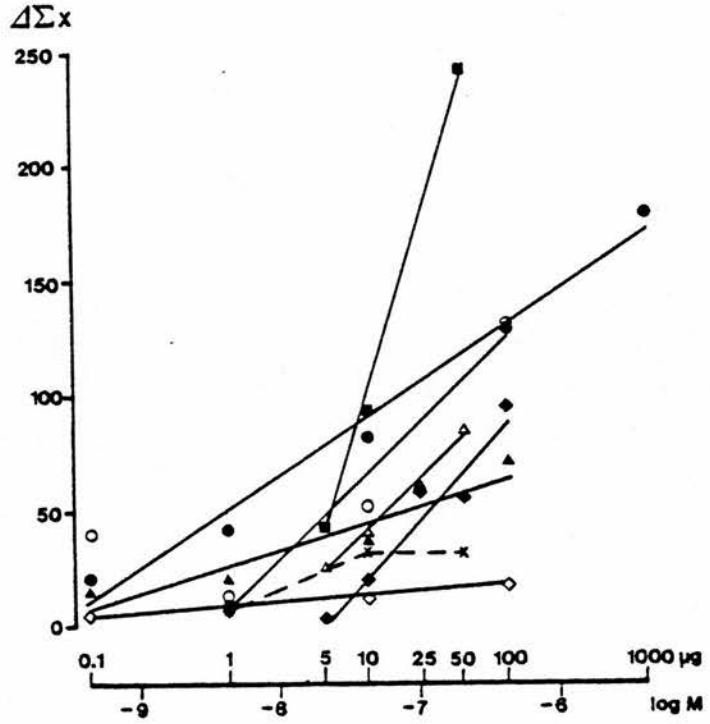


Figure 3.19. Eight sets of dose response data for the integrated response of chemoreceptors, in separate experiments, to i.c. injection of SAL, plotted on the same axes. Lines were fitted to the data by the method of least squares.

Figure 3.20. Chemoreceptor responses to SAL recorded in two separate experiments (A and B): both recordings were of multiple units, and data are plotted against dose.

1. Chemoexcitation integrated with respect to pre-injection control.

2. Mean discharge during chemoexcitation expressed as a percentage change from pre-injection control.

3. Δmax, showing no clear dependency upon dose.

4. Duration of the response (in seconds).

5. Mean discharge during pre-injection control (open symbols) and during drug-evoked excitation (closed symbols) showing the general progressive increase in background rate of discharge, and the relatively small, constant increase in discharge evoked by SAL.

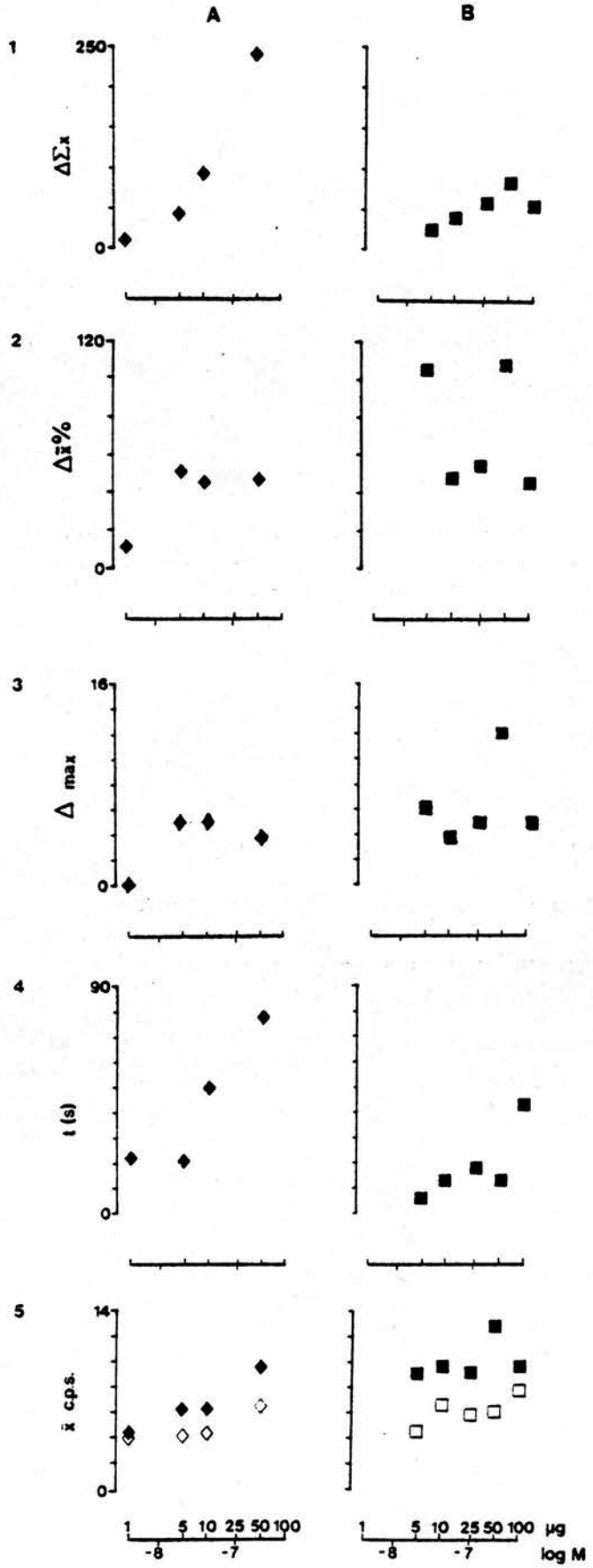

A
B
1
250
0
ΔΣx
2
120
0
Δx̄%
3
16
0
Δ max
4
90
0
t (s)
5
14
0
x̄ c.p.s.
1 5 10 25 50 100
-8 -7
1 5 10 25 50 100 µg
-8 -7 log M

Figure 3.21. A. Integrated responses of chemoreceptors to SAL (1-100 µg i.c.) before (O) and after (●) ICI 118551 (100 µg kg^{-1}, i.c.). The antagonist clearly reduced the excitatory response to the drug.

B. Δmax in the same experiment, showing no clear dose-relationship either before, or after the antagonist, but an obvious reduction in magnitude following ICI 118551.

C. Integrated response of chemoreceptors to injection of SAL (5-500 µg i.c.) in a separate experiment, before (O) and after ICI 118551 100 µg kg^{-1} (●) and 1000 µg kg^{-1} (◆), showing no attenuation , but perhaps a slight potentiation of the response. After injection of MET (100 µg kg^{-1}; □) chemoexcitation evoked by SAL was markedly attenuated.

D. Δmax for the same responses as those presented in (C), showing only slight dose-dependency, and slight reduction of the effect by ICI 118551, and a much larger reduction after MET.

'L' denotes the effect of injecting Locke solution, 0.3 ml i.c. The straight lines fitted to the data were determined by the method of least squares.

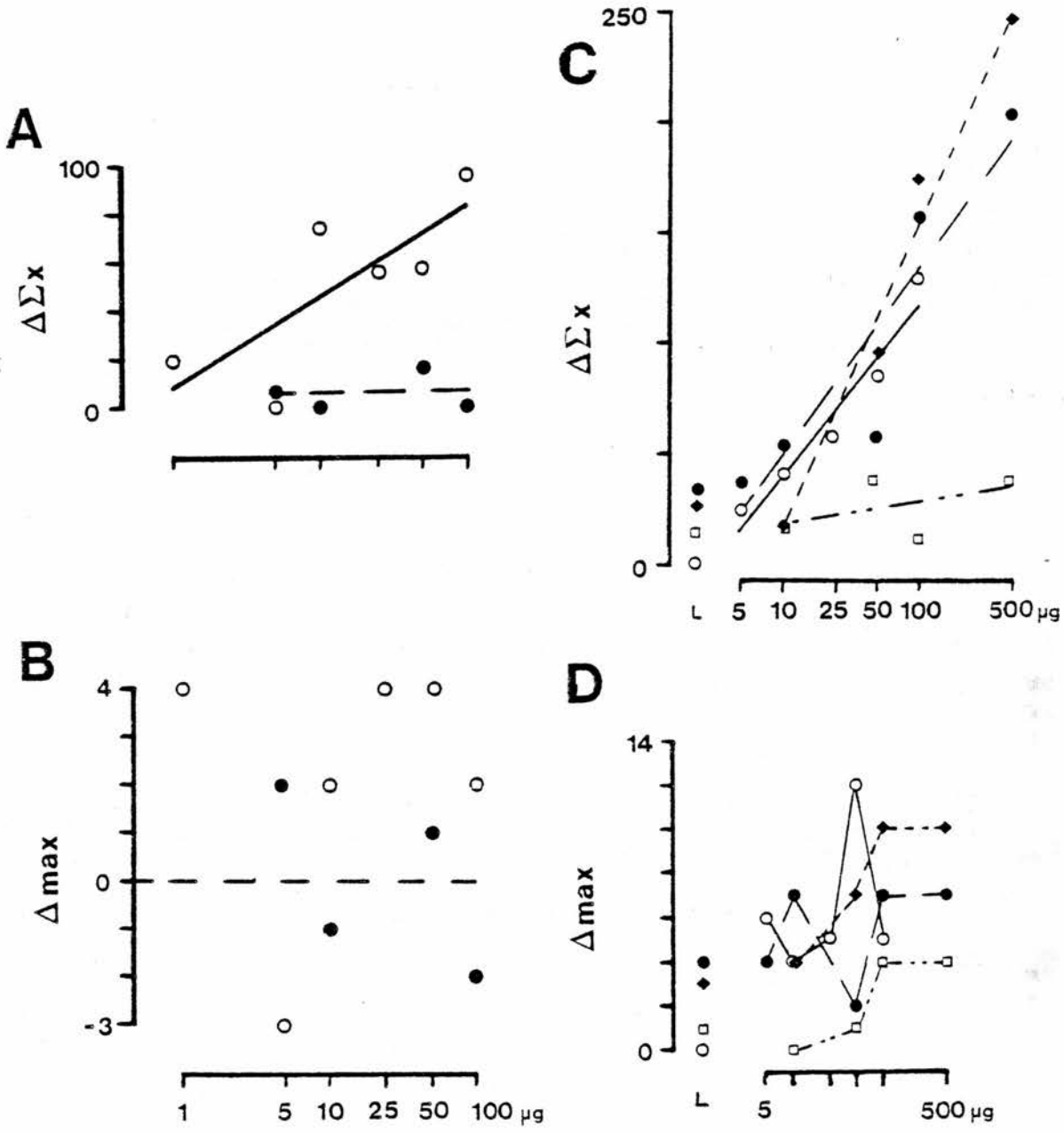
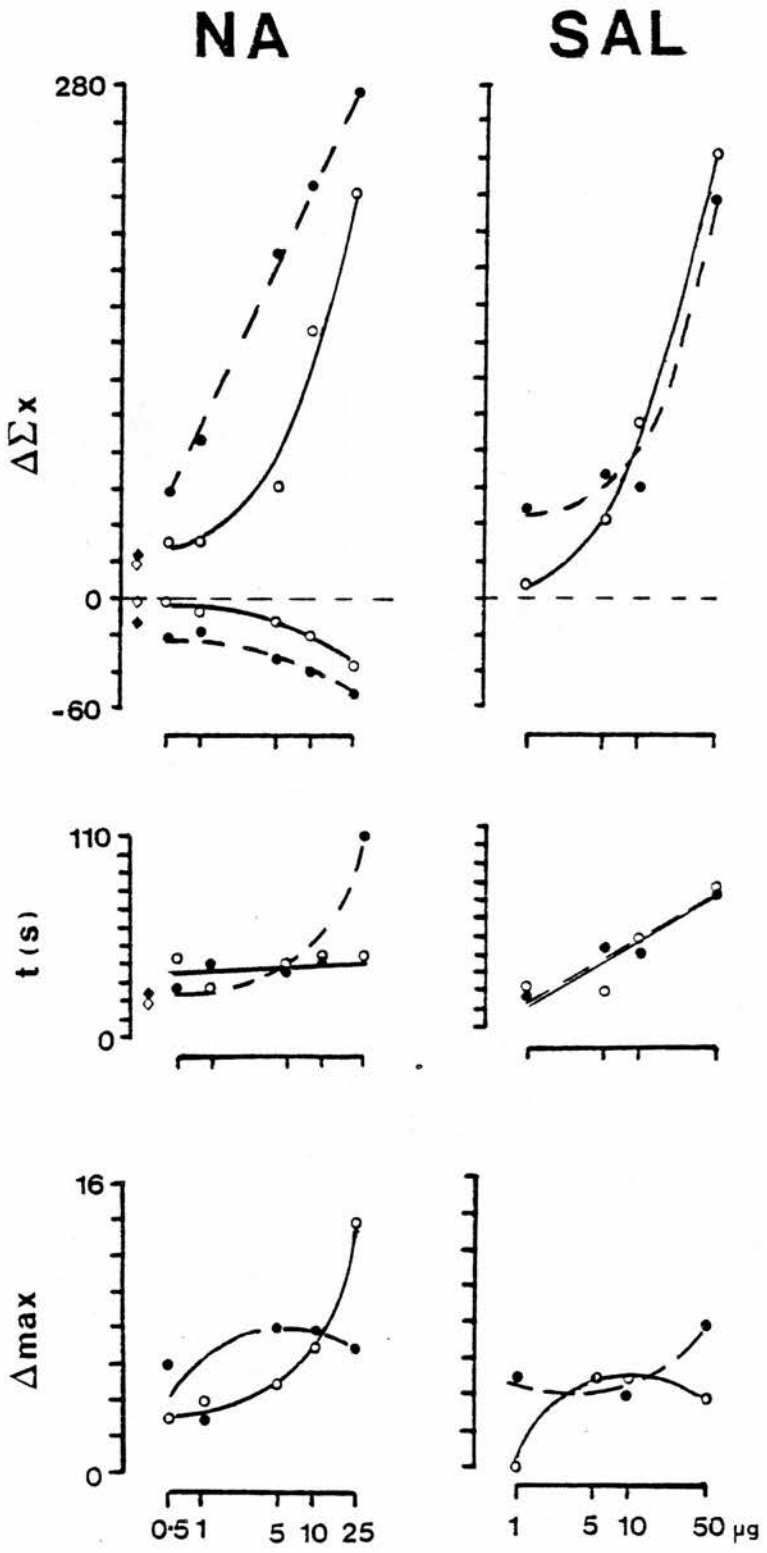

A
100
0
ΔΣx
C
250
0
ΔΣx
5 10 25 50 100 500 µg
B
4
0
-3
Δmax
1 5 10 25 50 100 µg
D
14
0
Δmax
5 500 µg

Figure 3.22. Dose response data from a single experiment for i.c. injections of NA (0.5-25 μg) and SAL (1-50 μg), before (O) and after (●) ICI 118551 (100 μg kg^{-1}). Chemodepression was evoked only by NA, and both parts of the integrated response to NA were potentiated by the antagonist, whilst the integrated response to SAL was not markedly altered.
◊ or ◆ denotes the effects of injecting Locke solution, 0.3 ml.

Duration of the response [t(s)] was approximately log-linearly related to dose in the case of SAL, and remained unchanged by the antagonist, despite a reduction in the hypotensive effects of SAL after ICI 118551; the duration of NA-evoked excitation was not related to dose before the antagonist, but appeared slightly more so after administration of ICI 118551.

Δmax in the case of NA-evoked excitation was exponentially related to dose before ICI 118551, and increased after the antagonist, with a reduction in the maximum discharge attained. With SAL this parameter was more constant, and less clearly changed by the antagonist.


NA
SAL
280
0
-60
ΔΣx
110
0
t (s)
16
0
Δmax
0·5 1 5 10 25
1 5 10 50 µg

also reduced SAL-evoked chemoexcitation, either alone (MET; 4.11 mg kg^{-1} in cumulative doses), or following ICI 118551 (Fig. 3.21 C,D). Where the chemoexcitatory response to SAL was initially reduced by BET the effect was not further attenuated by administration of ICI 118551. Chemoexcitatory responses to SAL that persisted in the presence of β-receptor blockade were generally comparable to the E_1-type responses associated with NA (e.g. Fig. 3.26).

In the four experiments where the effects of NA upon chemoreceptor discharge were studied before and after injecting ICI 118551 this antagonist only slightly affected the responses evoked by NA. There was a slight reduction in the chemoexcitatory response to NA in two experiments (Fig. 3.23), and a small potentiation of the response in the other two (e.g. Fig. 3.22). Subsequent administration of β_1-antagonists caused no further attenuation of the response in two experiments. Injection of either MET or BET in two other experiments was followed by a loss of chemoexcitatory responses to NA (e.g. Fig 3.23), together with a potentiation of the initial depressant effect.

3.2.8 Persistance of chemoexcitation after adrenoceptor blockade.

A degree of chemoexcitation (notably the E_1-type) appeared to persist in the presence of combinations of adrenoceptor antagonists (e.g. Figs 3.24, 3.25) in ten experiments, as shown in table 3.2.

E_1-excitation in response to injecting 10 μg doses of NA was studied more closely in one experiment where it was found to be largely unaltered by raising or lowering arterial pH; it was initially reduced after injection of BET (100 μg kg^{-1} i.c.), but subsequently was not markedly changed after injecting, in turn, ICI 118551 0.1 and 1 mg kg^{-1}, RAU 0.1 mg kg^{-1}, COR 0.1 μg kg^{-1}, mecamylamine 1 mg

Table 3.2: Details of antagonists used, after which there was still an excitatory response to the injection of noradrenaline.

Expt	Sequence of antagonists, and doses (mg kg^{-1})
1	ICI 118551, 0.1; BET, 0.1
2	DP, 0.1; MET, 0.001, 0.01, 0.1, 1; ICI 118551, 0.1
3	DP, 0.1; BET, 0.001, 0.01, 0.1, 1; ICI 118551, 0.1; COR, 0.1
4	BET, 0.01, 0.1; RAU, 0.1; BET, 1; ICI 118551, 0.1; RAU, 1
5	MET, 0.1; DP, 0.1; MET, 1, 3.4; RAU, 1; PROP, 1.7; COR, 1
6	BET, 0.01, 0.1, 1; RAU, 0.1; COR 0.3 + RAU 0.3
7	ICI 118551, 0.1; RAU, 1; BET 0.1, 1; PROP 5.8
8	ICI 118551, 0.1, 1; MET 0.1
9	DP 0.01; MET 1; RAU, 1; ICI 118551, 0.35; COR, 1
10	BET 0.1; ICI 118551, 0.1, 1; RAU, 0.1; COR, 0.1; Mecamylamine & Atropine

Figure 3.23. Responses of 2-3 chemoreceptor units to i.c. injection of NA 1-50 μg before (O) and after ICI 118551 (1 mg kg^{-1}; ●) and BET (100 μg kg^{-1}; ▲).

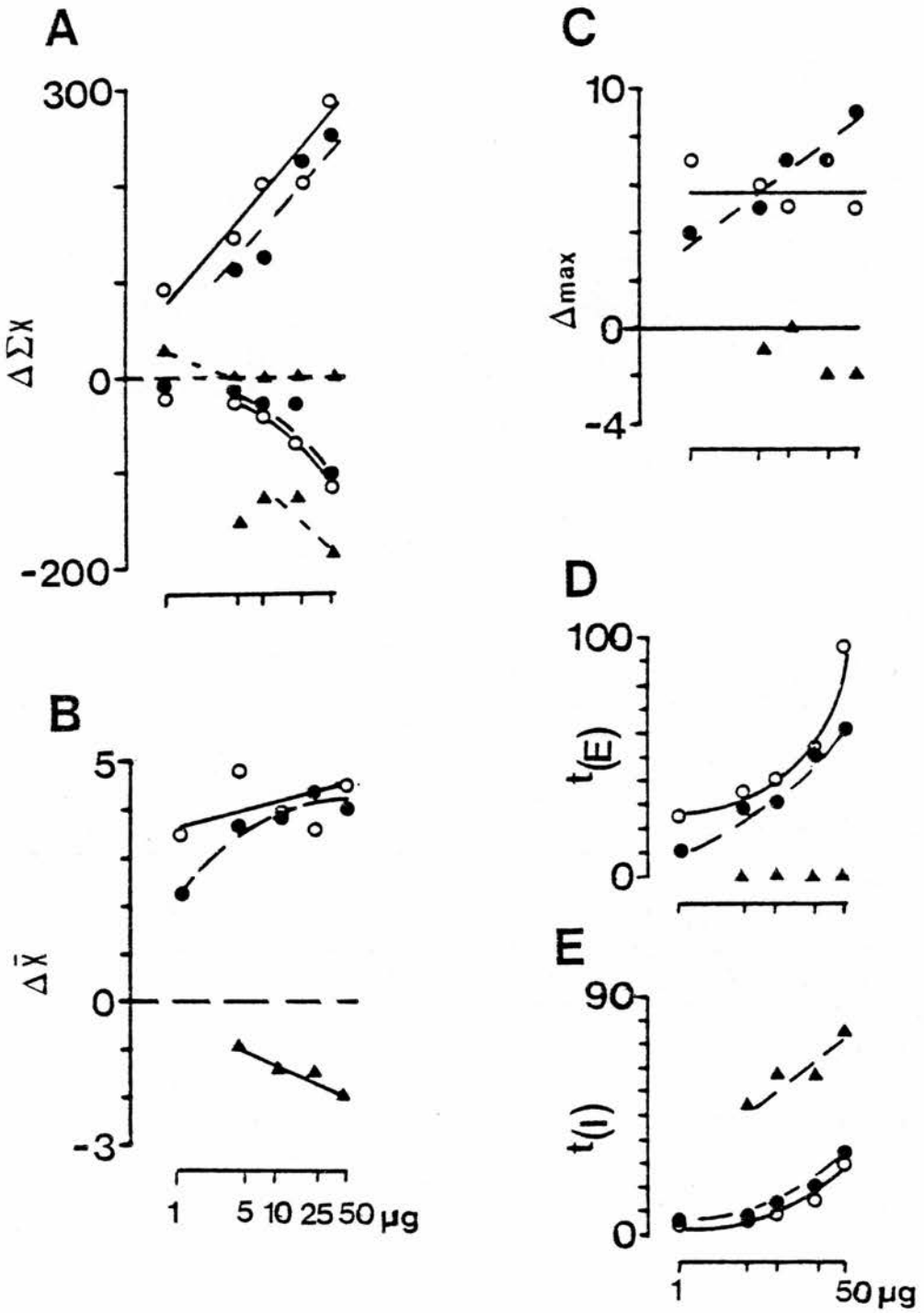
A. The integrated response ($\Delta\Sigma x$) plotted against dose; chemodepression was markedly potentiated after BET, whilst the chemoexcitatory response was abolished.

B. $\Delta\bar{x}$ for the excitatory effect of NA plotted against dose; there was a slight reduction after ICI 118551, and this form of analysis yielded negative values after BET, when comparing delayed chemoexcitation with pre-injection control.

C. Δmax, which was enhanced after ICI 118551, becoming a dose-dependent function; after BET the ability of NA to cause an increase in discharge was no longer apparent.

D. Duration (s) of the excitatory response to NA.

E. Duration (s) of the chemodepression evoked by NA; this was clearly increased after BET.


A
300
0
-200
ΔΣχ
B
5
0
-3
Δχ̄
1
5 10 25 50 µg
C
10
0
-4
Δmax
D
100
0
t(E)
E
90
0
t(I)
1
50 µg

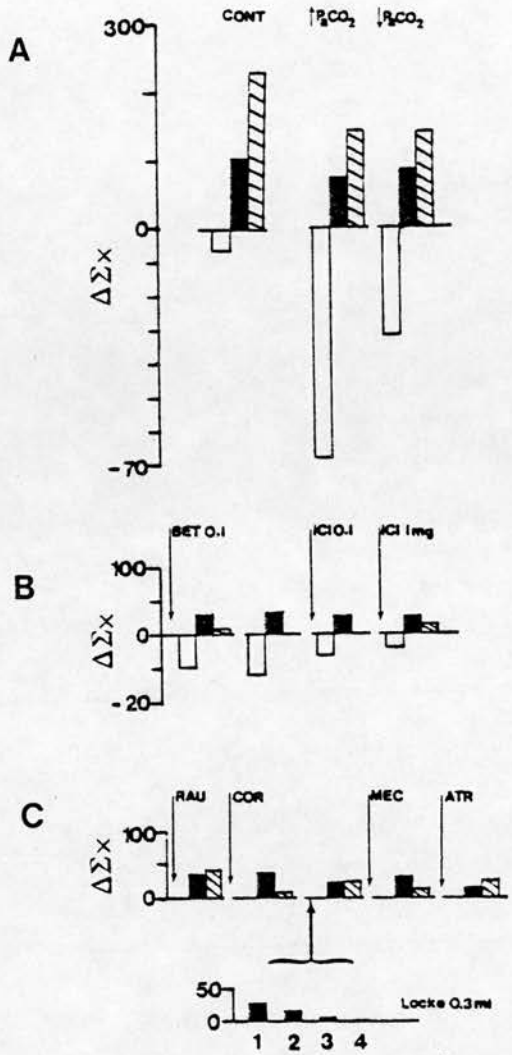


Figure 3.24. A. Responses to injections of NA 10 µg i.c., under control conditions and after increasing and reducing once again $PaCO_2$. Initial chemodepression is denoted by the open bars, E_1-excitation by the solid bars, and E_2-excitation by the hatched bars.

B. Responses to NA 10 µg after BET 100 µg kg^{-1}, ICI 118551 0.1 and 1 mg kg^{-1}, showing the persistence of E_1-type excitation.

C. Responses to NA 10 µg after RAU (0.1 mg kg^{-1}), COR (0.1 mg kg^{-1}), mecamylamine (MEC; 1 mg kg^{-1}), and atropine (ATR). E_1-excitation was present in response to each injection, and was not markedly variable in magnitude. Inset below are the effects of four injections of Locke solution (0.3 ml i.c.), where the progressive reduction of an excitatory response to injection of vehicle (between two doses of NA causing E_1-excitation) suggests that the effect is not artefactual.

Figure 3.25. Records of end-tidal CO_2 (CO_2; calibration 0-5.5%), femoral arterial blood pressure (BP 0-200 mm Hg) and rate of chemoreceptor discharge (proportional analogue signal - 0-10 c.p.s.) in a single experiment.

A. Responses to NA, 1-10 µg i.c., before antagonists.

B. Responses to SAL, 1-50 µg i.c., before antagonists.

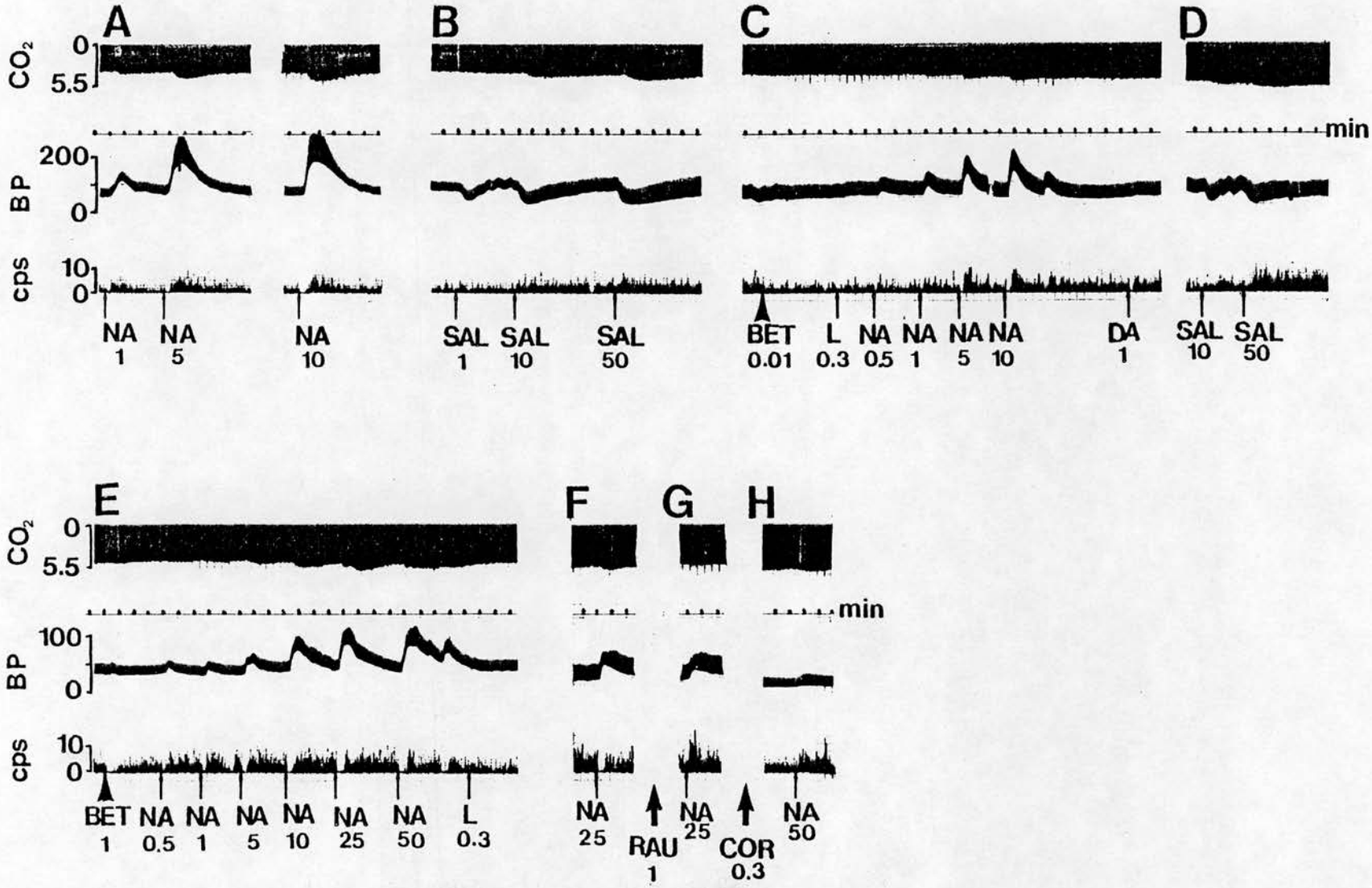
C. Effects of injecting BET 0.01 mg kg^{-1}, which attenuated inotropic and chronotropic responses to injected NA (0.5-10 µg i.c.). Effects of injecting Locke solution (L 0.3 ml) were negligible, and the response to DA (1 µg) was not radically different from pre-antagonist control (not shown).

D. Response to SAL, 10 and 50 µg, after the low dose of β_1-antagonist.

E. Effects of injecting BET, 1 mg kg^{-1} i.c., after which NA (0.5-50) caused prolonged depression of chemoreceptor discharge, an effect not associated with injection of Locke solution (L 0.3 ml) i.c.

F. NA 25 µg i.c., before, and after (G) RAU 1 mg kg^{-1}, where chemodepression was abolished, and replaced by a more prominent excitation of chemoreceptor discharge.

H. Response to NA after injecting COR 0.3 mg kg^{-1} (after the other antagonists); vascular responses to NA injection were virtually abolished, whilst some chemoexcitation persisted.


A
B
C
D
CO_2
0
5.5
BP
200
0
cps
10
0
min
NA 1
NA 5
NA 10
SAL 1
SAL 10
SAL 50
BET 0.01
L 0.3
NA 0.5
NA 1
NA 5
NA 10
DA 1
SAL 10
SAL 50
E
F
G
H
100
BET 1
NA 0.5
NA 1
NA 5
NA 10
NA 25
NA 50
L 0.3
NA 25
RAU 1
NA 25
COR 0.3
NA 50

kg^{-1}, and a large unknown dose of atropine (Fig. 3.24). Following COR four injections of Locke solution (0.3 ml) were made; with the first there was an obvious E_1-chemoexcitatory response, which was not observed with the third and fourth such injections (see Fig. 3.24). Subsequently, 10 µg NA caused E_1-excitation as previously, suggesting the effect is not an injection artefact. Before injection of BET there was prominent E_2-excitation, but this was no longer such a marked effect after this, or subsequent antagonists.

3.2.9 Comparison of the route of injection.

In two experiments a comparison was made of the effects of NA injected close to the carotid body (i.c.), or distally, by injecting into the femoral artery or femoral vein. Similar injections of NaCN and DA were made in one experiment for comparison. Results are shown in table 3.3. Chemodepression was not observed when NA was injected i.v. or i.a., but chemoexcitation occured whichever route of injection was used, although the duration, latency, and intensity varied.

3.2.10 Cardiovascular effects of injected adrenoceptor agonists and antagonists.

The monitoring of arterial blood pressure and gas tensions, E.C.G, and end-tidal CO_2 was performed routinely to assess the state of the animal during the experiment. These measurements also provided evidence of the biological activity or relative potency of a particular drug where effects upon chemoreceptor activity were equivocal.

Injections of more than 1 to 5 µg of NA caused a dose-related increase in systemic arterial blood pressure, with a latency of some 20 s (Fig. 3.26). Heart rate (cf. Figs 3.27 and 3.28) and pulse

Table 3.3: Comparison of the effects of NA, DA, and NaCN injected intracarotidally (i.c.), intravenously (i.v.), & intra-arterially (i.a).

	i.c.					i.v.					i.a.				
	$-\Delta\bar{x}\%$	$+\Delta\bar{x}\%$	d	t	Δm	$-\Delta\bar{x}\%$	$+\Delta\bar{x}\%$	d	t	Δm	$-\Delta\bar{x}\%$	$+\Delta\bar{x}\%$	d	t	Δm
NA															
10 µg	-89	96	48	22		0	86	27	125		-	-	-	-	-
NA															
1µg	-65	0	--	--	0	0	27	3	17	1	0	23	4	23	1
5µg	-81	29	14	40	3	0	49	12	43	3	0	20	1	49	-
10µg	-86	56	21	79	3	0	66	15	50	4	0	21	1	102	3
NaCN															
5 µg	0	1050	2	13	43	0	25	6	47	1	0	0	0	0	0
50 µg	-	-	-	-	-	0	25	9	21	0	0	24	1	71	1

DA	$-\Delta\bar{x}\%$	d	t	$-\Delta\bar{x}\%$	d	t	$-\Delta\bar{x}\%$	d	t
1µg	-85	0	16	-8	8	25	0	0	0
10 µg	-	-	-	-	-	-	0	0	0
50 µg	-	-	-	-	-	-	-16	9	19

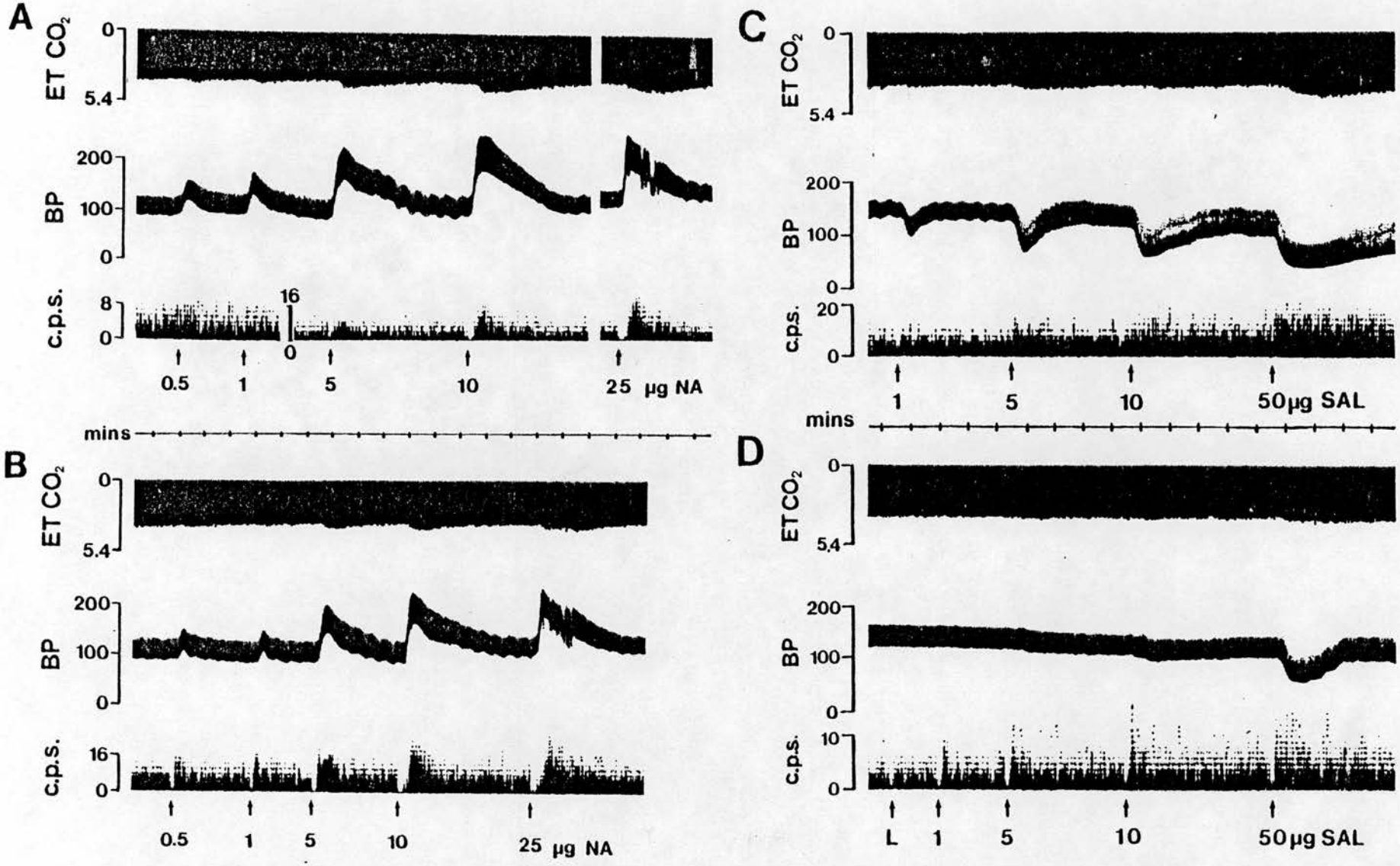
$-\Delta\bar{x}\%$: $\Delta\bar{x}\%$ for chemodepression; $+\Delta\bar{x}\%$: $\Delta\bar{x}\%$ for chemoexcitation; d: delay (s) before chemoexcitation, or, in the case of DA, chemodepression; t: duration (s) of chemoexcitation (or, for DA, chemodepression); Δm: Δ max.

Figure 3.26. A: The effects upon end-tidal CO_2 (ET CO_2, 0-5.4%), femoral arterial blood pressure (BP, 0-200 mmHg), and chemoreceptor discharge (cps - analogue signal proportional to discharge rate - 0-8 and 0-16 counts per second) of injecting NA (0.5, 1, 5, 10, 25 µg i.c. at arrows).

B: Effects of injecting the same doses of NA after administration of the β_2-adrenoceptor antagonist ICI 118551, 100 µg kg^{-1}.

C: The effects upon end-tidal CO_2, femoral arterial blood pressure, and chemoreceptor discharge (cps - analogue signal proportional to discharge rate - 0-10 and 0-20 counts per second), in the same experiment, of injecting SAL (1, 5, 10, 50 µg i.c. at arrows).

D: Effects of injecting the same doses of SAL, and Locke solution (0.3 ml), after administration of the β_2-adrenoceptor antagonist, ICI 118551, 100 µg kg^{-1}.


A
ET CO2
0
5.4
BP
200
100
0
c.p.s.
8
0
16
0
0.5
1
5
10
25 µg NA
mins
B
ET CO2
0
5.4
BP
200
100
0
c.p.s.
16
0
0.5
1
5
10
25 µg NA
C
ET CO2
0
5.4
BP
200
100
0
c.p.s.
20
0
1
5
10
50µg SAL
mins
D
ET CO2
0
5.4
BP
200
100
0
c.p.s.
10
0
L
1
5
10
50µg SAL

pressure were also increased (Fig. 3.26); end tidal CO_2 increased concomitant with the peak increase in blood pressure following each injection of NA (Fig. 3.26). The higher doses of NA were often associated with heart arrythmias, effectively limiting the upper range of doses that could be applied.

3.2.10.1 Effects of α_1-selective agonists and antagonists.

In nine cats PHEN (1 µg - 2 mg i.c.) caused a marked, dose-related increase in systemic arterial blood pressure which was substantially reduced, or more usually abolished, by the antagonist COR.

COR itself (0.1-1 mg kg^{-1} i.c.; n=4) caused no discernable changes in the tracing of femoral arterial blood pressure. The pressor response to NA was similarly attenuated in three (75%), and unaltered in one (25%) out of four experiments. The antagonism of NA-evoked pressor responses by COR was generally less than its antagonism of the hypertensive effects of the more selective agonist PHEN.

3.2.10.2 Effects of α_2-selective agonists and antagonists.

OXM (0.1-200 µg i.c.) injected in five cats caused a pronounced, dose-dependent increase in blood pressure which was consistently reduced or abolished by the antagonist RAU (0.1 or 1 mg kg^{-1}).

The antagonist RAU (0.01-4 mg kg^{-1}, i.c.) was injected in ten experiments. Doses of 100 µg kg^{-1} or greater often caused severe hypotension (mean blood pressure fell to ~50-60 mm Hg or less), which was not readily reversed by injection of 2.5% dextran-5% glucose solution. In four of the experiments the chemoreceptor recording was lost very soon after administering the antagonist, and in at least one experiment hypotension was the immediate cause of death. The antag-

onist blocked pressor responses to injected OXM, and in all nine experiments where examined, NA-evoked increases in blood pressure were substantially reduced after the antagonist.

In the presence of COR and RAU together changes in systemic arterial blood pressure were no longer seen to occur after injection of NA (Fig. 3.25). Although NA caused hypertension and changes in heart rate, neither of the α-selective agonists was associated with changes in heart rate.

3.2.10.3 Effects of β-selective agonists.

In twelve cats ISO (β_1/β_2) caused dose-dependent hypotension, increased pulse pressure, and tachycardia; the duration of these effects was similarly dose-dependent.

The β_1-selective agonist DOB was studied in four experiments, in three of which no discernable changes in the cardiovascular or respiratory parameters monitored occured after injecting DOB over the range 1-100 µg i.c. or (n=1) 5 mg i.v. In the fourth experiment, where pHa was near the upper limit of normal (>7.40), DOB (100 µg i.c.) caused a slight but transient rise in blood pressure.

As with DOB, no changes in cardiovascular or respiratory activity were detected after injecting prenalterol (PREN) in three cats over the range 0.1 µg - 1 mg i.c., or, (n=1) 5 mg i.v. (Fig. 3.13).

Single injections, or the injection of a range of doses of SAL (0.1 µg -1 mg i.c.) in thirteen cats caused systemic hypotension, an increase in arterial pulse pressure, an increase in end-tidal CO_2, and chemoexcitation. Blood pressure changes were dose-dependent with respect to magnitude and duration (Fig. 3.26), and were reduced or abolished by the antagonist ICI 118551 (0.1 or 1 µg kg^{-1}; cf. Fig.

3.26) in the six experiments where this combination of drugs was studied. There appeared to be a direct temporal relationship between blood pressure effects, changes in end-tidal CO_2, and chemoexcitation.

In one experiment, as blood pressure returned to normal following SAL injection, chemoreceptor discharge stabilised to a new level, approximately double the rate of discharge in the pre-injection control period. In the new steady-state, after SAL, PaO_2 was 75 mm Hg; $PaCO_2$, 23 mm Hg, and pHa, 7.35. These data could be compared with results of blood gas analysis performed some 40 min before injecting SAL when PaO_2 was 85 mm Hg, $PaCO_2$, 19 mm Hg, and pHa, 7.43.

3.2.10.4 Effects of β-selective antagonists.

MET (β_1-selective) was injected over a range of 0.1 μg - 1 mg kg^{-1} i.c. in thirteen cats, and caused a reduction in arterial blood pressure, pulse pressure, and resting heart rate. End-tidal CO_2 tended to decrease slightly with the decrease in blood pressure, from which there was some degree of recovery after about five minutes.

Table 3.4A shows the results of blood gas analyses performed before and after the administration of MET. Only PaO_2 was significantly altered (increased) as a result of injecting MET, but the effect is possibly time-dependent, as shown in table 3.4B.

This antagonist reduced the NA-evoked increase in heart rate in a dose-dependent manner (Fig. 3.27[1]) although, following initial low doses of the antagonist (Fig. 3.27[2]), basal heart rate (recorded before injections of NA) was significantly higher than the same measurements made before injecting MET.

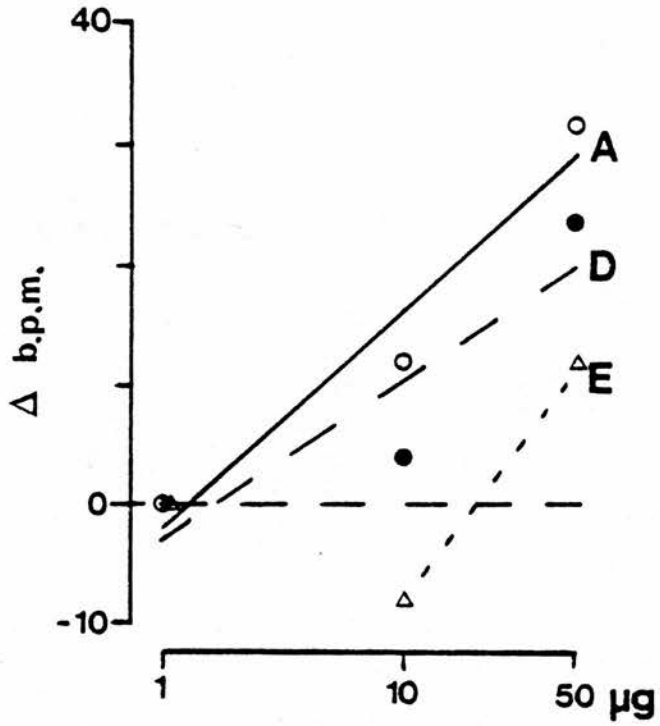
BET was studied in ten experiments; doses of 100 μg kg^{-1} or more caused a fall in blood pressure, a short lasting reduction of end-

Figure 3.27. 1. Cardiac acceleration (Δ b.p.m. - $\bar{x}$ beats per minute at time of peak increase in blood pressure minus $\bar{x}$ beats per minute in pre-injection control period) plotted as a function of dose of NA, before (A - O) and after MET 10 (D - ●) and 100 (E - Δ) μg kg^{-1}.

2. Mean heart rate before (A; n=4) and after met 0.1 (B; n=3), 1 (C; n=3), 10 (D; n=4), and 100 (E; n=4) μg kg^{-1}. Mean heart rate was calculated in the control period before individual injections of NA, at each stage of the experiment. * = statistically different from control ($P<0.05$ - Student's t-test).

Lines were fitted to the data using the method of least squares.

1



2

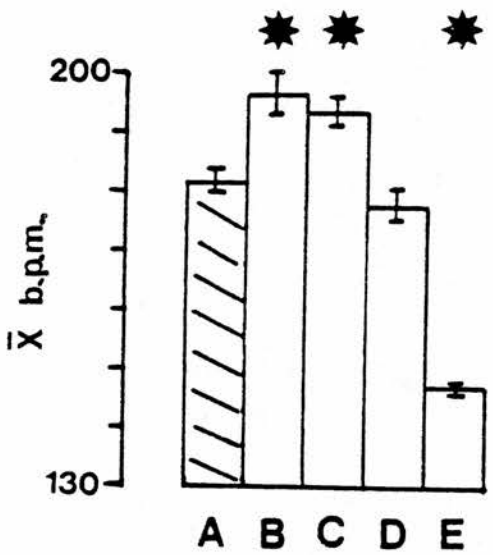


Table 3.4: A: PaO_2, $PaCO_2$, and pHa before and after metoprolol; B: results of blood gas analyses in a single experiment taken before, five, and eleven minutes after injecting metoprolol 0.1 mg kg^{-1}. $\Delta\bar{x}$ represents the mean difference between results obtained before and after the drug.

A.

PaO_2 (mm Hg)		$PaCO_2$ (mm Hg)		pHa		
Control	MET	Control	MET	Control	MET	Dose (kg^{-1})
74	70	44	44	7.22	7.21	0.1 µg
64	67	46	49	7.17	7.17	1 µg
82	93	-	-	7.36	7.36	10 µg
81	89	38	38	7.22	7.26	10 µg
87	91	36	35	7.25	7.28	0.1 mg
110	112	35	36	7.23	7.25	0.1 mg
90	95	30	21	7.38	7.37	0.5 mg
67	82	47	27	7.25	7.31	3.1 mg
77	75	39	38	7.24	7.26	3.1 mg
$\Delta\bar{x}$	5.0		-3.4		0.02	
s.e.m.	2.2		2.7		8×10^{-3}	
	P<0.05		P>0.1		P>0.05	(paired t-test)

B.

	CONTROL	MET (0.1 mg kg^{-1})	After 5'	After 11'
PaO_2 (mm Hg)	93	128	115	101
$PaCO_2$ (mm Hg)	29	29	27	27
pHa	7.36	7.37	7.38	7.36

tidal CO_2, reduced pulse pressure, and bradycardia. Cardiovascular effects of lower doses of BET (1-10 μg kg^{-1}) were only very slight, but the tachycardia and increased pulse pressure elicited by injected NA were reduced following doses of BET as low as 10 μg kg^{-1} (figure 3.28). Blood gas analyses carried out before and after injection of BET show no significant changes (paired t-test) in PaO_2, $PaCO_2$, or pHa (table 3.5).

The β_2-selective antagonist ICI 118551 was injected in ten experiments. At a dose of 0.1 or 1 mg kg^{-1} it caused slight hypertension, a reduction in pulse pressure, and no change, or a slight increase or small decrease in end-tidal CO_2. The most marked and consistent effect of the antagonist, even at the lower dose of 100 μg kg^{-1}, was the almost complete blockade of hypotension caused by injecting SAL (Fig. 3.26) or infusing ISO. It did not appear to alter the cardiovascular response to NA (Fig. 3.26). Blood gas analyses carried out before and after injection of the antagonist are shown in table 3.6.

3.2.10.5 Effects of catecholamine-induced changes in blood pressure upon arterial blood gas tensions.

In a few cases blood samples were taken before and after changes in blood pressure to determine whether there was any change in blood gas tensions that might correlate with the increased chemoreceptor discharge that occured concomitant with both hyper- and hypo- tensive effects of adrenoceptor agonists. Samples were taken when the change in blood pressure evoked by a drug was maximal, and in two cases a second sample was taken immediately after the first. Differences in values obtained before and after changes in blood pressure were

Figure 3.28. 1. Cardiac acceleration (Δ b.p.m. - $\bar{x}$ beats per minute at time of peak increase in blood pressure minus $\bar{x}$ beats per minute in pre-injection control period) plotted as a function of dose of NA before (A) and after BET 1 (B), 10 (C), and 100 (D) μg kg^{-1}. Lines were fitted to the data using the method of least squares.

2. Before BET, SAL (50 μg i.c.) caused tachycardia (A); the effect was reduced by BET 1 μg kg^{-1} (B) and 10 μg kg^{-1} (C), with little further attenuation of the response after BET 100 μg kg^{-1}.

3. OXM (100 μg kg^{-1}) elicited very little increase in heart rate (A), which was not obviously detectable after BET.

4. Mean heart rate before (A; n=4) and after BET 1 (B; n=5), 10 (C; n=5), 100 (D; n=4) kg^{-1}. Mean heart rate was calculated in the control period before individual injections of NA, at each stage of the experiment. * = statistically different from control ($P<0.05$ - Student's t-test).

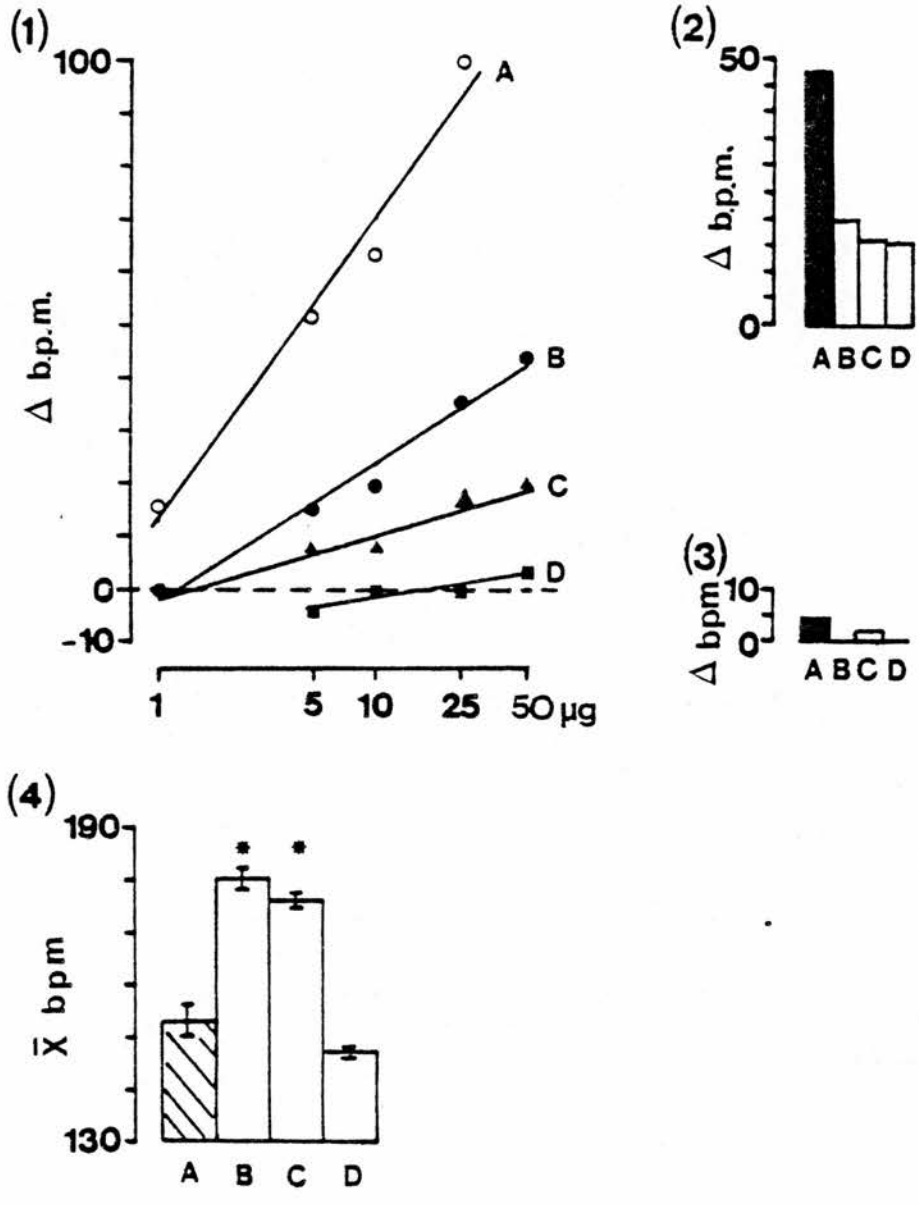

(1)
100
0
-10
Δ b.p.m.
A
B
C
D
1
5
10
25
50 µg
(2)
50
0
Δ b.p.m.
A B C D
(3)
10
0
Δ bpm
A B C D
(4)
190
130
X̄ bpm
A
B
C
D

Table 3.5: PaO_2, $PaCO_2$, and pHa before and after betaxolol.
$\Delta\bar{x}$ represents the mean difference between results obtained before and after the drug.

PaO_2 (mm Hg)		$PaCO_2$ (mm Hg)		pHa		
Control	BET	Control	BET	Control	BET	Dose (kg^{-1})
106	115	--	--	7.12	7.20	0.1 mg
103	111	33	31	7.21	7.15	0.2 mg
77	79	26	27	7.29	7.31	10 µg
61	58	27	28	7.30	7.31	1 µg
129	134	29	31	7.26	7.26	10 µg
124	120	31	31	7.26	7.23	0.1 mg
120	116	31	32	7.23	7.25	1 mg
80	78	30	32	7.23	7.20	0.1 mg
83	82	24	23	7.20	7.27	5 mg
72	77	33	32	7.21	7.23	0.1 mg
68	76	33	30	7.20	7.23	1 mg
64	77	30	32	7.22	7.24	2.12 mg
$\Delta\bar{x}$	3.0		0.2		0.01	
S.E.M.	1.7		0.5		0.01	
	P=<0.05		P=<0.2		P= <0.4	(paired t-test)

Table 3.6: PaO_2, $PaCO_2$, and pHa before and after ICI 118551.
$\Delta\bar{x}$ represents the mean difference between results obtained before and after the drug.

PaO_2 (mm Hg)		$PaCO_2$ (mm Hg)		pHa		
Control	ICI	Control	ICI	Control	ICI	Dose (kg^{-1})
89	106	30	32	7.16	7.15	100 µg
76	78	29	28	7.32	7.36	100 µg
104	120	34	33	7.24	7.24	1 mg
110	115	37	38	7.21	7.21	100 µg
$\Delta\bar{x}$	10.0		0.3		8×10^{-3}	
S.E.M.	3.8		0.8		0.01	
	P=<0.05		P=>0.05		P= >0.05	(paired t-test)

compared using a paired t-test; results are shown in table 3.7.

Supplementation of anaesthetic was not accompanied by a change in discharge frequency, although there was a rapid but short-lasting fall in blood pressure. Changes in PaO_2, $PaCO_2$ and pHa were not significant ($P>0.05$).

At peak increase in blood pressure after injecting NA, PaO_2 fell ($0.1>P>0.05$); pHa was not significantly changed but the increase in $PaCO_2$ was significant at the 0.05 level. After injection of SAL both PaO_2 and $PaCO_2$ were increased, but the differences were not significant.

The effects of DOB and OXM were studied in one experiment each; DOB caused no changes in either PaO_2 or $PaCO_2$. PaO_2 at the peak of the blood pressure increase in response to OXM was increased and $PaCO_2$ was reduced, compared with pre-injection control.

3.3 Summary of results presented in section 3.

1. The cardiovascular effects of the adrenoceptor agonists and antagonists studied were consistent with those reported in the general literature, and were used as an index of biological activity of the drugs injected.

2. Dose-dependent depression of chemoreceptor discharge was caused by DA and NA; chemodepression evoked by DOB and PHEN was not clearly related to dose. Injections of OXM, PREN, ISO and SAL were not associated with chemodepression. The chemodepressant effect of both NA and DA was antagonised by the DA D_2-receptor antagonist domperidone, and to a lesser extent by the α_2-antagonist RAU. It was potentiated by the β_1-antagonists BET and MET.

3. Chemoexcitation was evoked by NA and OXM, and the effect was coincident with their hypertensive and cardiovascular effects.

Table 3.7: PaO_2, $PaCO_2$, and pHa before and at peak of drug-induced blood pressure changes.
$\bar{\Delta x}$ represents the mean difference between results obtained before and after the drug.

PaO_2 (mm Hg)		$PaCO_2$ (mm Hg)		pHa		
Before	After	Before	After	Before	After	Drug
99	100	36	38	7.27	7.23	PB†0.2 ml
92	105	39	34	7.28	7.30	PB 0.2 ml
$\bar{\Delta x}$	7.0		1.5		0.01	
S.E.M.	6.0		3.5		0.03	
	P=>0.05		P=>0.05		P=>0.05	(paired t-test)
PaO_2 (mm Hg)		$PaCO_2$ (mm Hg)		pHa		
Before	After	Before	After	Before	After	Drug
63	58	34	37	7.19	7.23	NA 50 µg
63	57 60	31	35 38	7.25	7.25 7.25	NA 50 µg
91	86	32	39	7.35	7.25	NA 50 µg
100	91 84	38	39 42	7.23	7.24 7.26	NA 50 µg
106	85	33	42	7.22	7.16	NA 25 µg
147	104	28	32	7.29	7.23	NA 10 µg
$\bar{\Delta x}$	-14.8		4.7		0.03	
S.E.M.	6.1		1.1		0.02	
	0.1>P>0.05		P=<0.05		P=>0.05	(paired t-test)
PaO_2 (mm Hg)		$PaCO_2$ (mm Hg)		pHa		
Before	After	Before	After	Before	After	Drug
116	126	32	35	7.25	7.22	SAL 50 µg
110	117	32	30	7.30	7.29	SAL 0.5 mg*
$\bar{\Delta x}$	8.5		0.5		-0.02	
S.E.M.	2.3		6.3		0.0001	
	P>0.05		P>0.05		P>0.05	(paired t-test)
PaO_2 (mm Hg)		$PaCO_2$ (mm Hg)		pHa		
Before	After	Before	After	Before	After	Drug (mg)
98	105	30	28	7.30	7.31	OXM 0.1
90	92	20	21	7.34	7.33	DOB 0.1

†=Pentobarbitone; *=after ICI 118551 1 mg kg^{-1}

Excitation caused by ISO and SAL was concomitant with the hypotensive and other cardiovascular effects resulting from injection of these drugs. PHEN, DOB, and PREN did not cause significant chemoexcitation at the doses studied.

4. An initial transient but intense excitatory response often occured in response to injection of NA or SAL, preceding the more 'diffuse' delayed excitatory response that appeared to be induced at the peak of the blood pressure change.

5. Delayed excitatory responses temporally associated with cardiovascular changes were generally reduced by appropriate α- or β-antagonists, either alone, or in combination. Some excitatory effects of NA, particularly early E_1-excitation, were potentiated after low doses of the β_1-antagonists BET and MET. However, the potentiation of chemodepression by these antagonists may complicate the quantitative analysis of this effect. E_1-excitation could persist after administration of α_1-, α_2-, β_1-, β_2-, and D_2-antagonists (and in one experiment mecamylamine and atropine also). There was evidence that the effect was not simply an injection artefact.

SECTION 4

EFFECTS OF INFUSIONS OF CATECHOLAMINES AND SELECTIVE ADRENOCEPTOR AGONISTS AND ANTAGONISTS, AND INTERACTIONS WITH HYPOXIA OR HYPERCAPNIA IN THE CAT

SECTION 4.

EFFECTS OF INFUSIONS OF CATECHOLAMINES AND SELECTIVE ADRENOCEPTOR AGONISTS, AND INTERACTIONS WITH HYPOXIA OR HYPERCAPNIA IN THE CAT.

4.1 Effects of infusing adrenoceptor agonists.

The infusion of a drug over a period of minutes should allow the study of the drug's effects in steady-state or equilibrium conditions, which do not exist following the injection of a bolus of the substance. Intracarotid infusions of catecholamine-related drugs were made at a rate of 0.1 ml min^{-1}, for up to 38 minutes. The mean discharge frequency was calculated in consecutive 15 s intervals; the mean steady-state discharge, when there are no longer any marked changes in the frequency of discharge, may be expressed in terms of a percentage change from the mean discharge in the pre-injection control period.

Chemoreceptor responses to infusion of NA (e.g. Fig. 4.1) were studied in eight experiments. Table 4.1 summarises the results obtained; in seven (88%), depression of discharge was elicited by NA at all doses studied. In one experiment (12%) infusion of NA 1-25 µg min^{-1} caused chemoexcitation, whilst infusion of greater amounts (50-100 µg min^{-1}) depressed discharge. Comparison between the columns of table 4.1 shows that the chemodepressant effect is not markedly dose-related, and comparison between rows shows that the effect was variable in magnitude from one experiment to another. Comparison of the depressant effects of infusion of NA 10 µg min^{-1} using a paired t-test shows a significant reduction in discharge, as compared with the

Table 4.1: Effects upon chemoreceptor discharge frequency of the infusion of NA. Mean frequency in steady-state conditions expressed as percentage change from pre-infusion control discharge.

Expt.	Locke (0.1ml min^{-1})	NORADRENALINE ($\mu g\ min^{-1}$) 1	5	10	25	50	100
1	+7	-	-	-52⊙	-	-	-
2	+12.5	-14	-154†	-	-	-	-
3	-13	-	-	-50⊙	-	-	-
4	+9	-	-	-50⊙	-	-	-
5	0	-	-42†	-80⊙	-50†	-29	-
6	+9	-	-4†	-82⊙	-28†	-	-
7	+7	-92	-97†	-	-	-	-
8	+20	+33	+40	+45⊙	+50	-92	-20
$\bar{x}$	+6.4	-24.3	-51.4	-44.8*	-9.3	-60.5*	-20
s.e.m.	3.4	36.5	34.1	19.0	30.3	31.5	-
n	8	3	5	6	3	2	1

* = $P<0.05$, with respect to control (non-paired t-test).
† = $0.1>P>0.05$, ⊙ = $P<0.05$ (paired t-test).

Table 4.2: Effects upon chemoreceptor discharge frequency of the infusion of ISO. Mean frequency in steady-state conditions expressed as percentage change from pre-infusion control discharge.

Expt.	Locke (0.1ml min^{-1})	ISOPRENALINE ($\mu g\ min^{-1}$) 1	5	10	50	100
1	+8	-17	-	-36*	-	+444
2	-	-	-	+30	-	-
3	+12	+200	+343	-	+150	-
4	-13	-	-	+63	-	-
	+11	-	-	+27	-	-
		-	-	-85*	-	-
5	-	-	-	+178	-	-
		-	-	+150	-	-
6	+9	-	-	+12.5	-	-
7	+9	-	-	-29*	-	-
$\bar{x}$	+6.0	+91.5	+343	+53.4	+150	+444
s.e.m.	3.8	108.5	-	24.6	-	-
n	6	2	1	9	1	1

(* = $0.1>P>0.05$, Student's paired t-test).

Figure 4.1. Data obtained in a single experiment showing the changes in chemoreceptor discharge, blood pressure and end-tidal CO_2 evoked during six minute infusions of adrenoceptor agonists.

1. Effects upon chemoreceptor discharge of infusing Locke solution (0.1 ml min^{-1}), and NA 5, 10, 25, and 50 µg min^{-1} for six minutes (solid bar).

Mean discharge (calibration: 0-10 c.p.s) has been calculated in successive 15 s intervals, and plotted against time. Pre-infusion control is denoted by filled blocks.

The effects of the infusions (indicated by the horizontal bars) upon end-tidal CO_2 (CO_2, calibration: 0-5%), and femoral arterial blood pressure (BP, calibration: 0-200 mm Hg) are shown on the right. (Note reduced time scale).

Results of blood gas analyses performed before (C) and at three and six minutes after onset of infusion are appended below.

Infusion (rate, min^{-1})	PaO_2 (mm Hg)			$PaCO_2$ (mm Hg)			pHa		
	C	3m	6m	C	3m	6m	C	3m	6m
Locke 0.3 ml	93	105	105	28.5	25.5	28.5	7.40	7.41	7.39
NA 5 µg	105	102	102	28.5	29.0	29.0	7.39	7.38	7.38
NA 10 µg	104	104	98	26.0	29.0	29.0	7.41	7.38	7.38
NA 25 µg	102	95	91	27.0	27.0	32.0	7.39	7.39	7.37
NA 50 µg	104	94	85	25.0	30.0	33.0	7.39	7.38	7.36

1

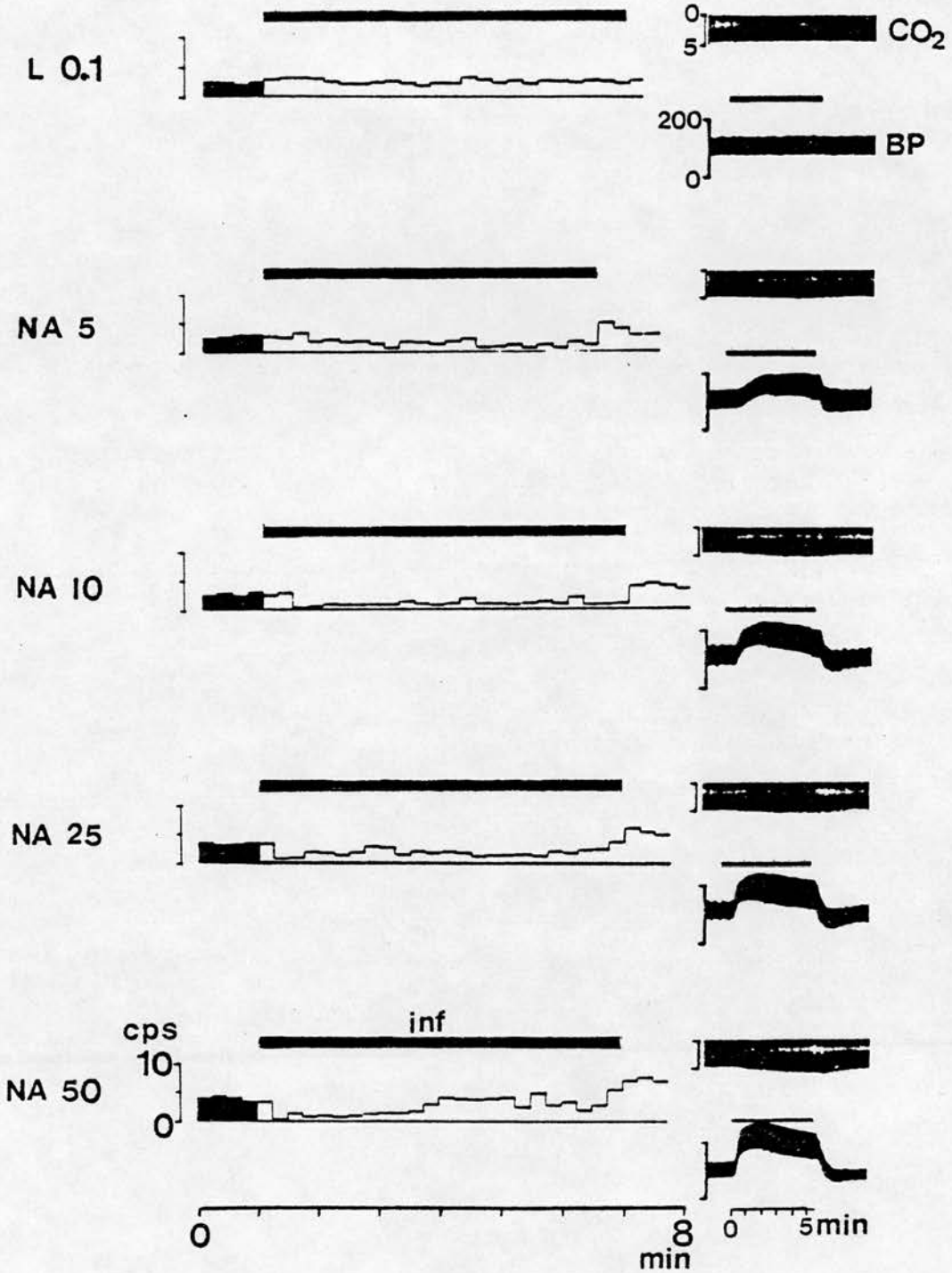


Figure 4.1. 2. Data obtained in a single experiment showing effects upon chemoreceptor discharge, end-tidal CO_2, and blood pressure, of infusing SAL 10 and 50 μg min^{-1}, OXM 10 and 100 μg min^{-1}, and DA 1 μg min^{-1}. Treatment of data is as described for figure 4.1 (1).

Results of blood gas analyses performed before (C) and at three and six minutes after onset of infusion are appended below.

Infusion (rate, min^{-1})	PaO_2 (mm Hg)			$PaCO_2$ (mm Hg)			pHa		
	C	3m	6m	C	3m	6m	C	3m	6m
SAL 10 μg	92	95	91	27.0	32.0	33.0	7.36	7.36	7.34
SAL 50 μg	78	81	82	29.0	29.0	30.0	7.34	7.36	7.34
OXM 10 μg	86	91	91	28.0	26.0	26.0	7.34	7.36	7.34
OXM 100 μg	99	94	102	22.0	28.0	23.5	7.35	7.37	7.38
DA 1 μg	94	94	93	27.0	26.0	28.0	7.36	7.33	7.34

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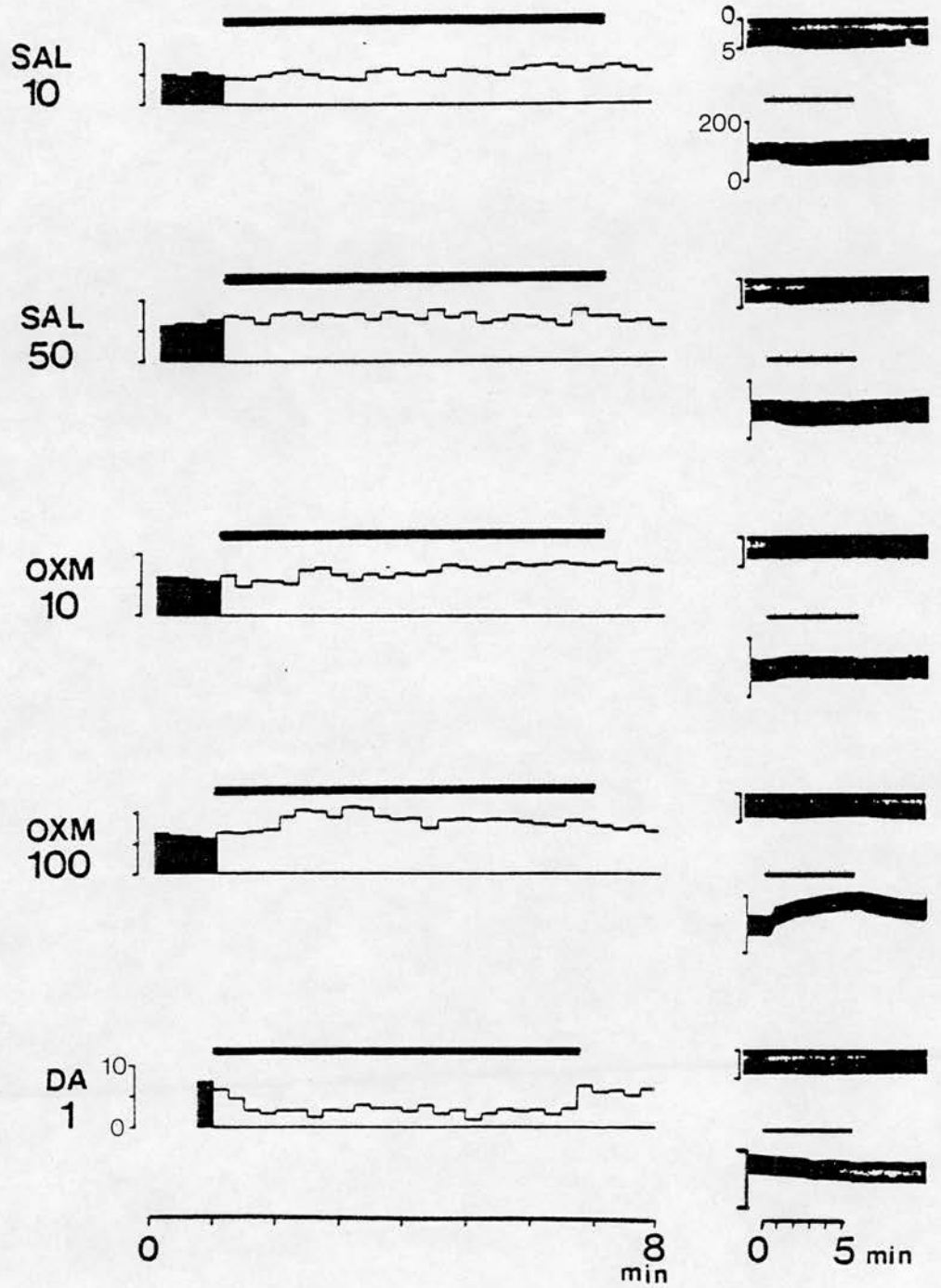


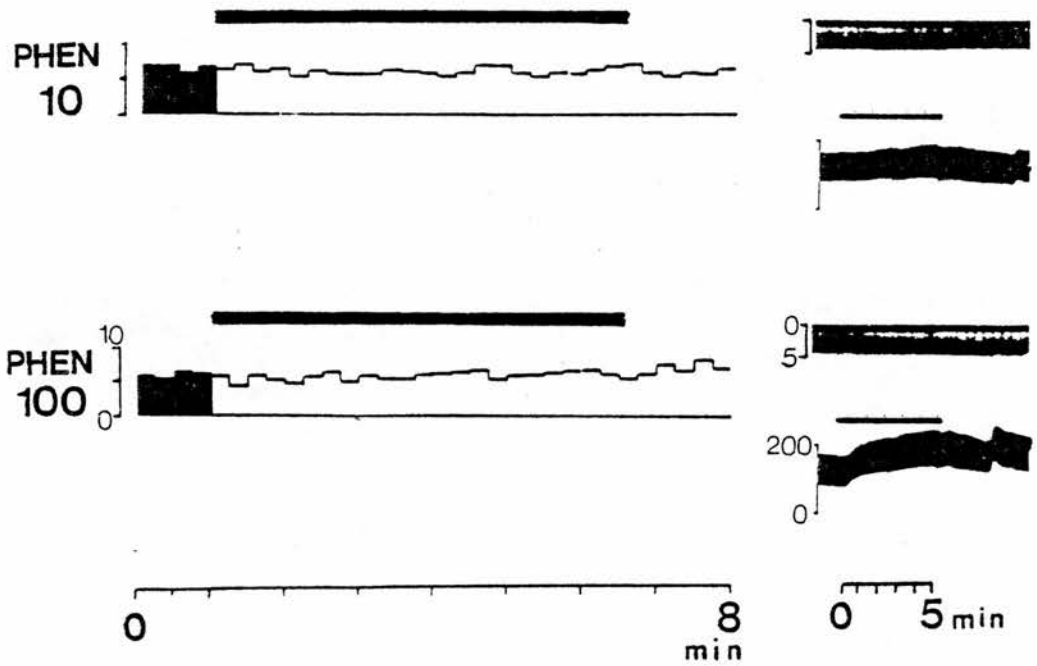


Figure 4.1. 3. Data obtained in a single experiment showing effects upon chemoreceptor discharge, end-tidal CO_2, and blood pressure, of infusing PHEN 10 and 100 μg min^{-1}; treatment of data is as described for figure 4.1 (1).

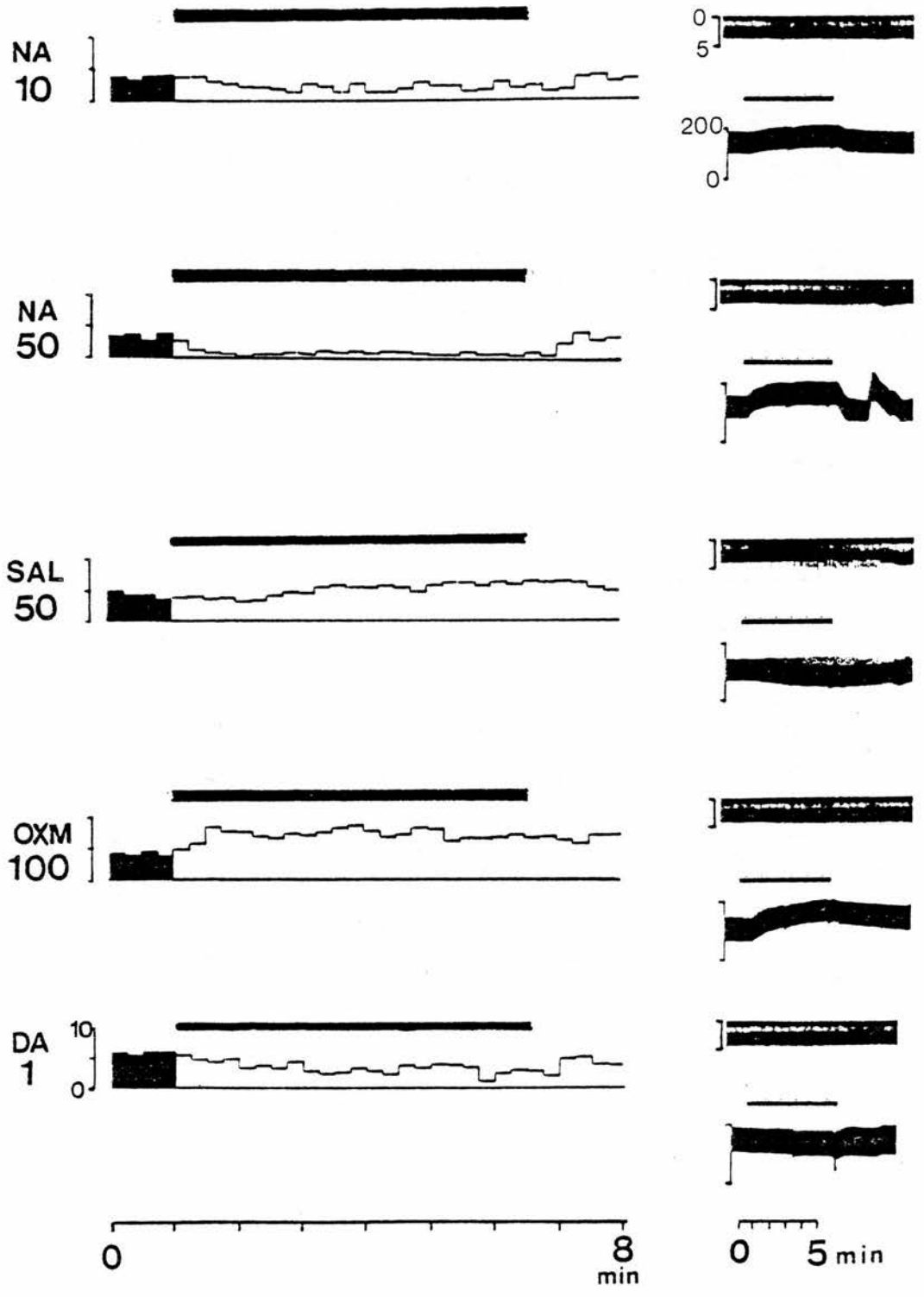
Results of blood gas analyses performed before (C) and at three and six minutes after onset of infusion are appended below.

Infusion	PaO_2 (mm Hg)			$PaCO_2$ (mm Hg)			pHa		
(rate, min^{-1})	C	3m	6m	C	3m	6m	C	3m	6m
PHEN 10 μg	86	90	89	28.0	23.0	26.0	7.34	7.36	7.36
PHEN 100 μg	89	88	86	26.0	26.0	26.0	7.36	7.38	7.38

Figure 4.1. 4. Data obtained in a single experiment showing effects upon chemoreceptor discharge, end-tidal CO_2, and blood pressure, of infusing NA 10 and 50 μg min^{-1}, SAL 50 μg min^{-1}, OXM 100 μg min^{-1}, and DA 1 μg min^{-1}, after injecting MET, 1 mg kg^{-1} i.c. Treatment of data is as described for figure 4.1 (1).

Results of blood gas analyses performed before (C) and at three and six minutes after onset of infusion are appended below.

Infusion (rate, min^{-1})	PaO_2 (mm Hg)			$PaCO_2$ (mm Hg)			pHa		
	C	3m	6m	C	3m	6m	C	3m	6m
NA 10 μg	93	91	99	24.0	25.0	25.0	7.39	7.38	7.37
NA 50 μg	89	94	93	24.5	23.0	25.0	7.38	7.42	7.40
SAL 50 μg	86	83	86	26.0	24.5	22.0	7.40	7.42	7.40
OXM 100 μg	91	88	92	22.5	26.0	24.0	7.39	7.40	7.39
DA 1 μg	91	85	89	26.0	26.5	26.0	7.39	7.38	7.39


after
MET 1mg kg⁻¹
NA
10
NA
50
SAL
50
OXM
100
DA
1
10
0
0
5
200
0
0
8
min
0
5 min

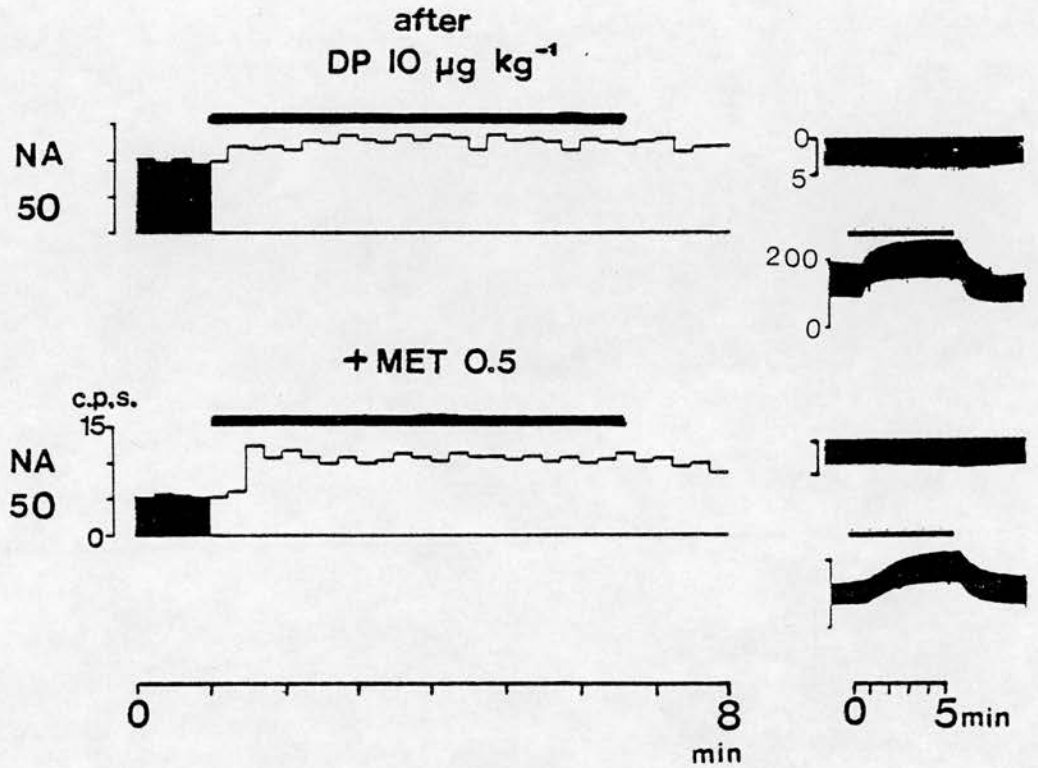


Figure 4.1. 5. Top: data obtained in a single experiment showing effects upon chemoreceptor discharge, end-tidal CO_2, and blood pressure, of infusing NA 50 µg min^{-1}, following injection of domperidone, 10 µg kg^{-1} i.c. (in the presence of MET, 1 mg kg^{-1}).

Bottom: data obtained in the same experiment showing effects upon chemoreceptor discharge, end tidal-CO_2, and blood pressure of infusing NA 50 µg kg^{-1}, after administering an additional 0.5 mg kg^{-1} MET i.c.

Treatment of data is as described for figure 4.1 (1).

Results of blood gas analyses performed before (C) and at three and six minutes after onset of infusion are appended below.

Infusion (rate, min^{-1})	PaO_2 (mm Hg)			$PaCO_2$ (mm Hg)			pHa		
	C	3m	6m	C	3m	6m	C	3m	6m
NA 50 µg (after domperidone 10 µg kg^{-1})	79	82	91	23.5	27.0	30.0	7.38	7.36	7.38
NA 50 µg (+MET 0.5 mg kg^{-1})	95	91	91	21.0	24.0	25.0	7.37	7.38	7.36

respective Locke infusion controls ($P<0.05$, $n=4$). Only two sets of data were significantly different from control when compared using a non-paired t-test, and including all data obtained.

The effects of MET (1 mg kg^{-1}, i.c.) upon chemoreceptor responses to NA infusion were examined in two experiments; MET reduced the chemodepression evoked by the lower level of NA infusion (10 µg min^{-1}) in both experiments, but potentiated chemodepression elicited by higher rates of NA infusion (Fig. 4.2).

Domperidone was studied in one experiment, after MET. A dose of 10 µg kg^{-1} greatly attenuated the MET-enhanced depressant effect of NA infusion (50 µg min^{-1}; Fig. 4.1(5)); after a further dose of MET (0.5 mg kg^{-1}) had been administered, infusion of NA (50 µg min^{-1}) caused a 30% increase in discharge (Fig. 4.1(5)).

ISO was infused i.c. in seven experiments and caused slight chemodepression in two (29%) but clear chemoexcitation in the other five (71%) experiments. Results are summarised in table 4.2 and, again, there was considerable variation in magnitude of the response between experiments, with no striking correlation between dose and response.

DA, infused in two experiments, caused chemodepression. In one of these experiments infusion of DA 1 µg min^{-1} for six minutes caused a progressive reduction in discharge with three distinct plateaux: there was a 13% reduction in discharge in the second minute of infusion, a 50% reduction in the fourth minute, and an 89% reduction by the sixth minute, with discharge returning rapidly to near control level upon cessation of the infusion (Fig. 4.3). Infusion of DA 10 µg min^{-1} in this experiment caused an initial 75% decrease in discharge, which was sustained over some two minutes, and discharge during the

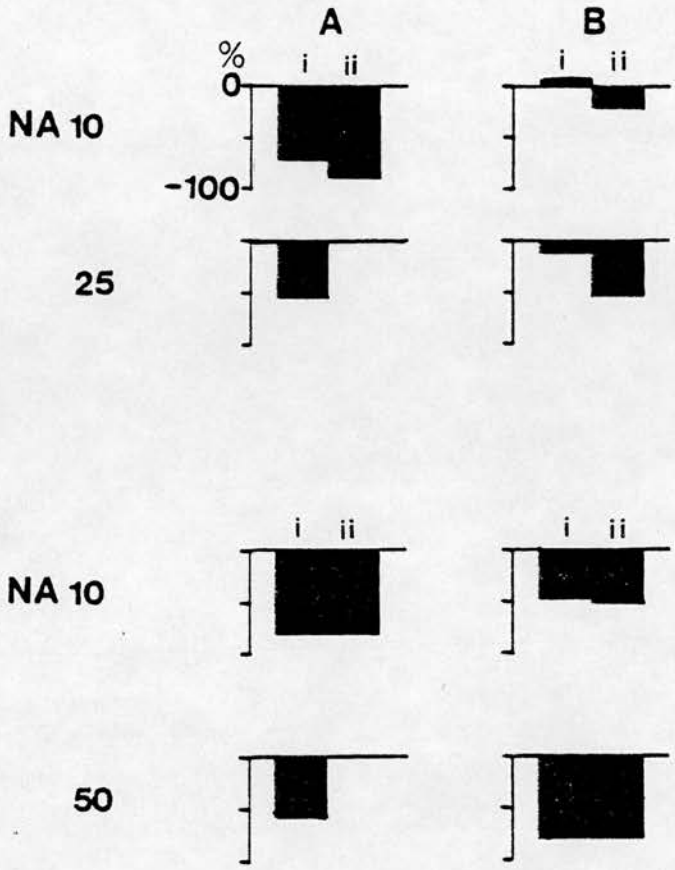


Figure 4.2. Results obtained in two separate experiments showing the effects of six minute infusions of (1) NA 10 and 25 and (2) NA 10 and 50 μg min^{-1} (A) before, and (B) After injecting MET, 1 mg kg^{-1}.

Mean discharge in the steady state attained in the first (i) and second (ii) three minutes of the infusions have been expressed as percentage changes from the mean discharge in the appropriate pre-infusion control (note that the initial depression of discharge by the higher doses was not sustained, before MET, and so, in the second three minutes of the infusion there was no net change in discharge as compared to control). These changes in steady-state discharge were apparent after taking arterial blood samples at the third minute of the infusion.

After injecting MET (B) the chemodepressant effect of infusing NA at a rate of 10 μg min^{-1} was reduced, whilst greater chemodepression was evoked at the higher rates of infusion, after the antagonist.

In both experiments, before the antagonist, discharge increased during infusion of NA at the higher rates after taking a blood sample at the third minute of infusion. During the remaining three minutes of infusion there was no net overall difference from discharge before starting infusion.

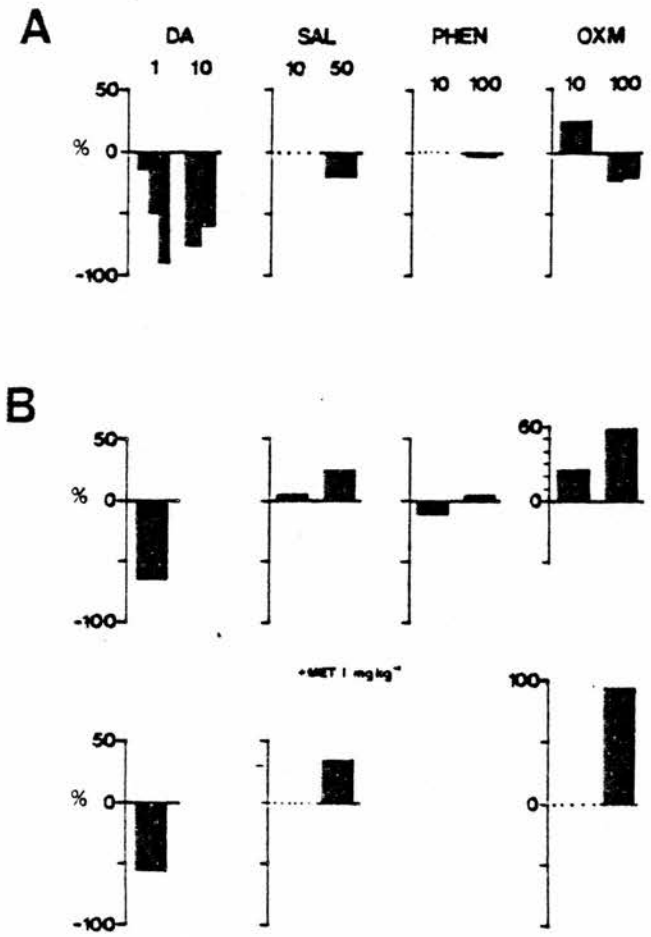


Figure 4.3. Comparison of the effects upon chemoreceptor discharge of infusing DA 1 and 10 μg min^{-1}, SAL 10 and 50 μg min^{-1}, PHEN 10 and 100 μg min^{-1}, and OXM 10 and 100 μg min^{-1} in two separate experiments, (A) and (B).

Discharge was calculated in succesive 15 s intervals and plotted against time; the mean discharge in steady-state conditions, expressed as a percentage change from the mean discharge in the respective pre-infusion control period is shown in this figure.

In the first experiment the chemodepression evoked by DA was progressive, and so two or three different degrees of reduced discharge are presented (see text). The steady-state discharge during infusion of OXM (100 μg min^{-1}) was slightly reduced after taking an arterial blood sample at the third minute of infusion. During all other drug-infusions a new steady-state level of discharge was rapidly attained after starting the infusion, and well sustained, and only a single percentage change is indicated.

In the second experiment (B) the effects of infusing DA 1 μg min^{-1}, SAL 50 μg min^{-1}, and OXM 100 μg min^{-1} were examined after injecting MET 1mg kg^{-1}. The depressant effect of DA infusion was slightly reduced, whilst the excitatory effects of infusing SAL or OXM were potentiated.

remaining four minutes of infusion remained constant at some 60% of control (Fig. 4.3). In the other experiment there was a 64% depression of discharge, within 30 seconds of onset of infusion, and the degree of depression remained constant throughout the period of infusion. After MET (1 mg kg^{-1}, i.c.), DA infusion (1 µg min^{-1}) caused only a 55% reduction in discharge.

SAL infusion caused slight but dose-related chemoexcitation in one experiment, with infusions of 10 and 50 µg min^{-1} causing 5% and 24% increases in discharge (at steady-state) respectively. When the infusion of SAL (50 µg min^{-1}) was repeated after MET (1 mg kg^{-1}, i.c.), it caused a 35% increase in discharge (Fig. 4.1). In the other experiment SAL (50 µg min^{-1}) caused an 18% reduction of discharge, this experiment being one of those in which infusion of ISO also caused depression.

The infusion of PHEN (n=2) had only minimal effects upon chemoreceptor discharge (e.g. Fig. 4.1). Thus, in one experiment, infusing 10 µg min^{-1} reduced discharge by some 11%, and increasing the rate to 100 µg min^{-1} resulted in a mere 4% increase in discharge; in a separate experiment infusion of PHEN 100 µg min^{-1} reduced discharge by 2.5% (Fig. 4.3).

In contrast, OXM was markedly more effective, causing a 25% increase in discharge frequency when infusing 10 µg min^{-1} (n=2; see Fig. 4.3). When the level of infusion was increased to 100 µg min^{-1} discharge was increased to 58% of control in one experiment but reduced by 22% in the other (a freshly prepared dilution of OXM similarly reduced discharge by 20% in the same experiment). In the experiment where the high dose of OXM caused only chemoexcitation the effect was potentiated after MET (1 mg kg^{-1}; Fig. 4.1, and Fig. 4.3).

Although in most instances infusion of a drug led to the rapid establishment of a new and stable steady-state of discharge, it was occasionally noticed that dishcarge could be separated into two distinct plateaux (e.g. NA 50 μg min^{-1} infusion, Fig. 4.1(1); Fig. 4.2). This change in steady-state, which sometimes resulted in the replacement of an excitatory effect by chemodepression, or vice versa, was seen to occur at the time of taking samples for arterial blood gas analysis at the third minute of infusion; during this procedure there was generally a transient rise fall and rise in arterial blood pressure.

Cardiovascular effects of the infusion of catecholamines and selective agonists were comparable to those elicited by injecting the same substances. Thus NA, PHEN, and OXM caused hypertension when infused, ISO and SAL, hypotension, and NA, ISO, and SAL infusions caused tachycardia. DA infusion was not associated with any clear changes in blood pressure or heart rate.

4.2 Interaction of ISO and changes in blood gas tensions likely to affect the chemoreceptors.

Because the purely excitatory effects of ISO infusions are probably less complex with respect to the underlying mechanism than is likely to be the case with NA (which produces more than one type of change in chemoreceptor activity) the effects of ISO were studied in more detail in three cats, and NA in one.

Infusion of ISO 10 μg min^{-1} i.c. caused hypotension, a marked reduction in PaO_2, and an increase in the rate of chemoreceptor discharge. In one experiment this excitation represented an increase of 150% and 178% over control discharge (Figs 4.4 and 4.6). In a

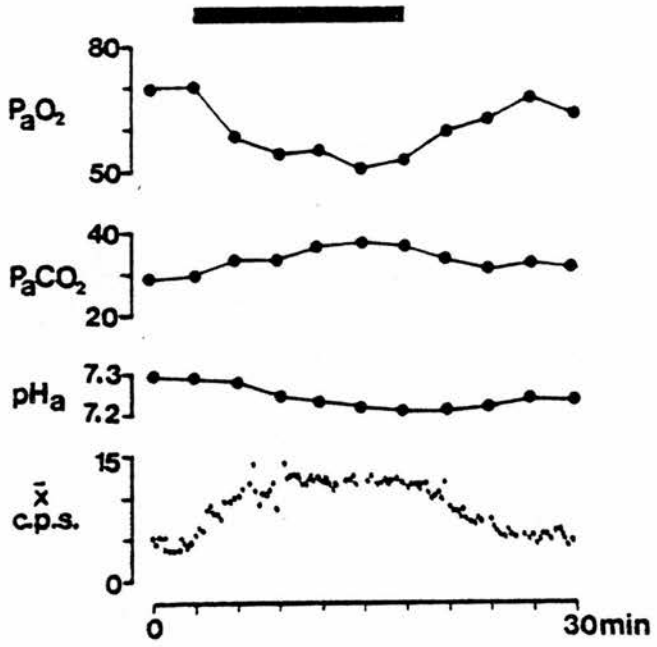


Figure 4.4. Data obtained in a single experiment showing effects upon PaO_2 (mm Hg), $PaCO_2$ (mm Hg), pHa, and frequency of chemoreceptor discharge ($\bar{x}$ c.p.s.) of infusing ISO (10 µg min^{-1}) for 15 minutes (solid bar). Chemoreceptor discharge was averaged in consecutive 15 s intervals. The animal was ventilated with room air, but the arterial oxygen tension throughout the experiment was consistent with mild hypoxia.

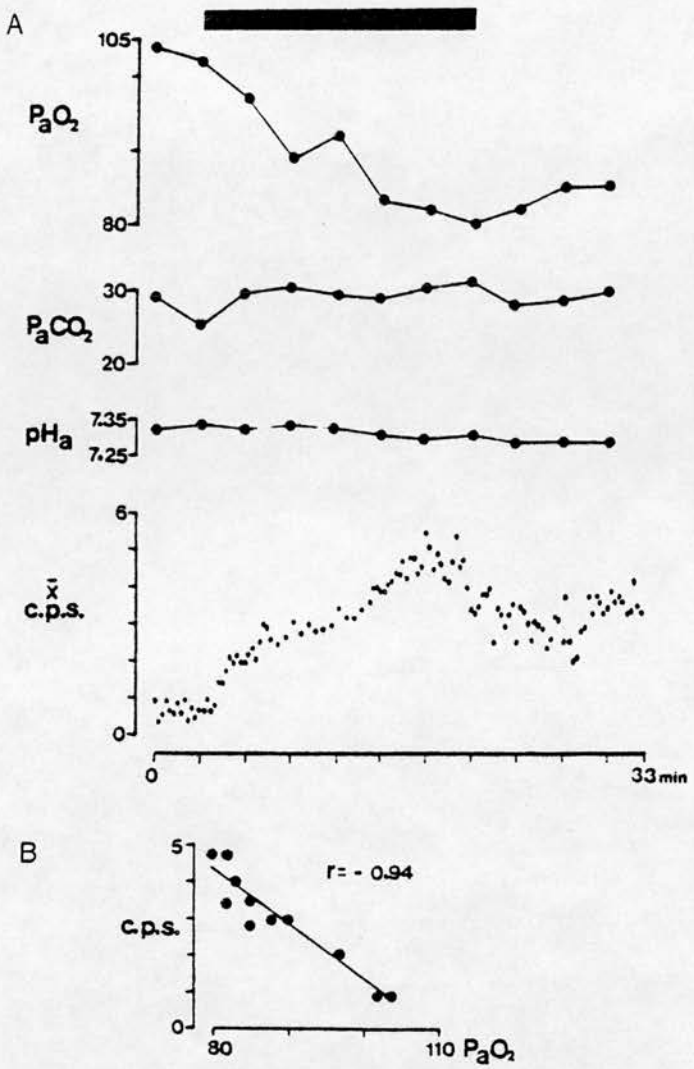


Figure 4.5. Data obtained in a single experiment showing (A) the effects upon PaO_2 (mm Hg), $PaCO_2$ (mm Hg), pHa, and frequency of chemoreceptor discharge ($\bar{x}$ c.p.s.) of infusing ISO (10 μg min^{-1}) for 18 minutes (solid bar). Chemoreceptor discharge was averaged in consecutive 15 s intervals. Heart rate during the pre-injection control period was 214 beats per minute (b.p.m.), rising to 242 b.p.m. (+13%) when the fall in blood pressure caused by ISO-infusion had stabilised, and to 247 b.p.m. (+15%) at the end of the infusion.

(B): Mean discharge (c.p.s.) has been plotted as a function of arterial oxygen tension (PaO_2; mm Hg); the straight line was fitted to the data points using the 'least-squares' method, and the correlation coefficient was -0.94.

separate experiment discharge was increased to 328% of control, rising further to 571% of control (Fig. 4.5); discharge in this case correlated very well with PaO_2 (Fig. 4.5).

The effects of hypercapnia in one experiment are shown in Figs 4.6 and 4.7. In the first example, at a relatively high level of PaO_2, ISO increased discharge concomitant with the fall in PaO_2; the addition of 1% and 3% CO_2 to the inspired gas caused further increases in discharge, to 183% and 250% of control, respectively. Although PaO_2 also decreased during the period of CO_2-breathing it did not fall below 100 mm Hg. Upon cessation of the infusion of ISO (10 μg min^{-1}), during CO_2-breathing, discharge decreased from 250% to 167% of control.

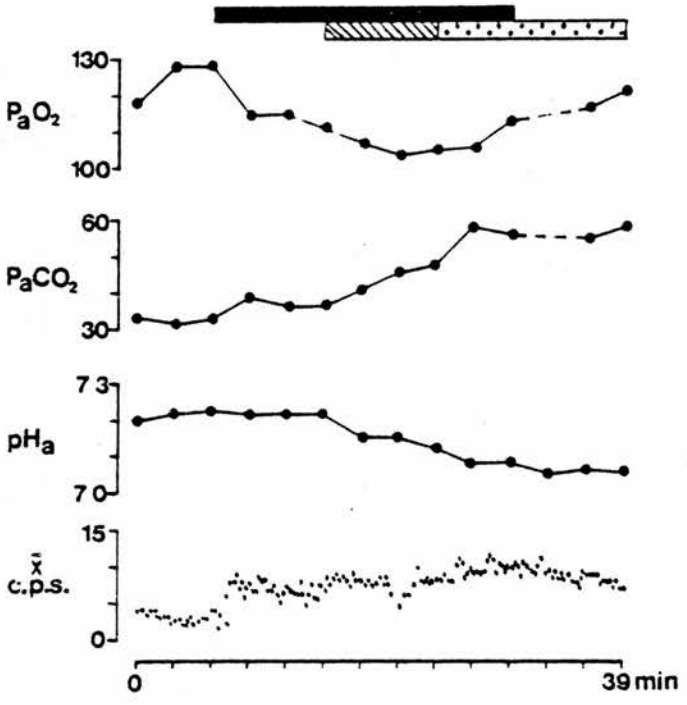
Under rather more hypoxic conditions (Fig. 4.7) ventilation with 1% CO_2 caused a 117% increase in discharge; infusion of ISO (10 μg min^{-1}) caused a further 61% increase in discharge which was then raised to 211% of control by increasing the inspired CO_2 to 3%.

Effects of hypercapnia in another experiment are shown in Fig. 4.8, for data obtained before and during infusion of ISO (10 μg min^{-1}). It was apparent (Fig. 4.9D) that the proportional response to CO_2 during infusion of ISO is not necessarily greater than in the absence of infusion when the percentage increase in discharge is related to the rate of discharge measured immediately prior to onset of hypercapnia.

In the third cat normoxia (PaO_2 110-120 mm Hg) largely suppressed the response of chemoreceptors to hypercapnia (Fig. 4.10). When PaO_2 was reduced and, more especially, during the infusion of ISO (10 μg min^{-1}; Fig. 4.10), the response to hypercapnia appeared to be restored. In this experiment the effects of NA infusion were also

Figure 4.6. Measurements of PaO_2 (mm Hg), $PaCO_2$ (mm Hg), pHa, and discharge frequency ($\bar{x}$ c.p.s. - averaged in consecutive 15 s intervals) recorded in a single experiment. ISO (10 μg min^{-1}) was infused for 24 minutes (solid bar), during which period CO_2 (1%) was added to the inspired gas (hatched bar) and then increased to 3% (stippled bar); ventilation with 3% added CO_2 was continued after stopping the infusion, and a slight fall in discharge rate was then apparent, allowing the estimation of ISO's contribution to the increased discharge.

Figure 4.7. Measurements of PaO_2 (mm Hg), $PaCO_2$ (mm Hg), pHa, and discharge frequency ($\bar{x}$ c.p.s. - averaged in consecutive 15 s intervals) recorded in a single experiment. 1% CO_2 was added to the inspired gas (hatched bar) and then raised to 3% (stippled bar); six minutes after the initial increase in inspired CO_2, ISO (10 μg min^{-1}) was infused for 22.5 minutes (solid bar), enhancing the increase in discharge evoked by CO_2.


130
P_aO_2
100
60
P_aCO_2
30
7 3
pH_a
7 0
15
$\bar{x}$
c.p.s.
0
0
39 min

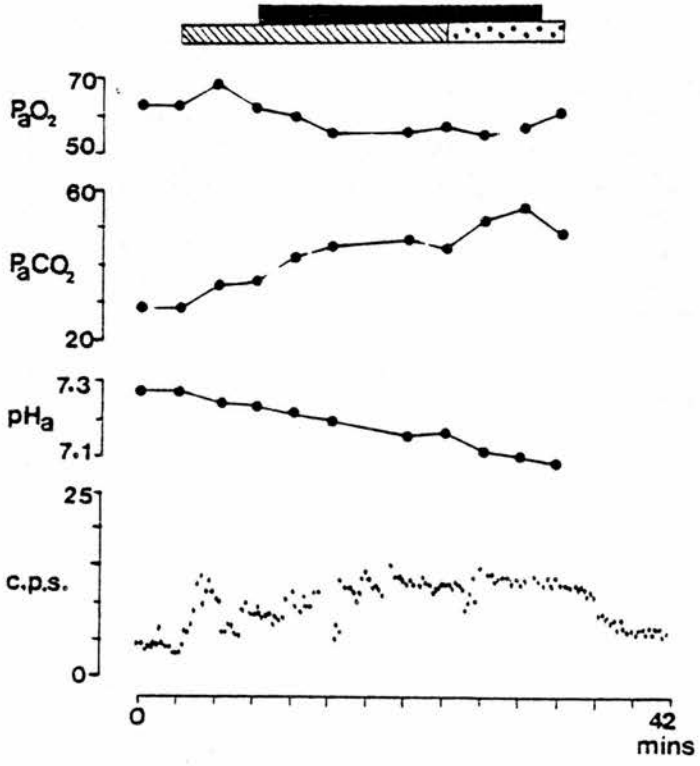
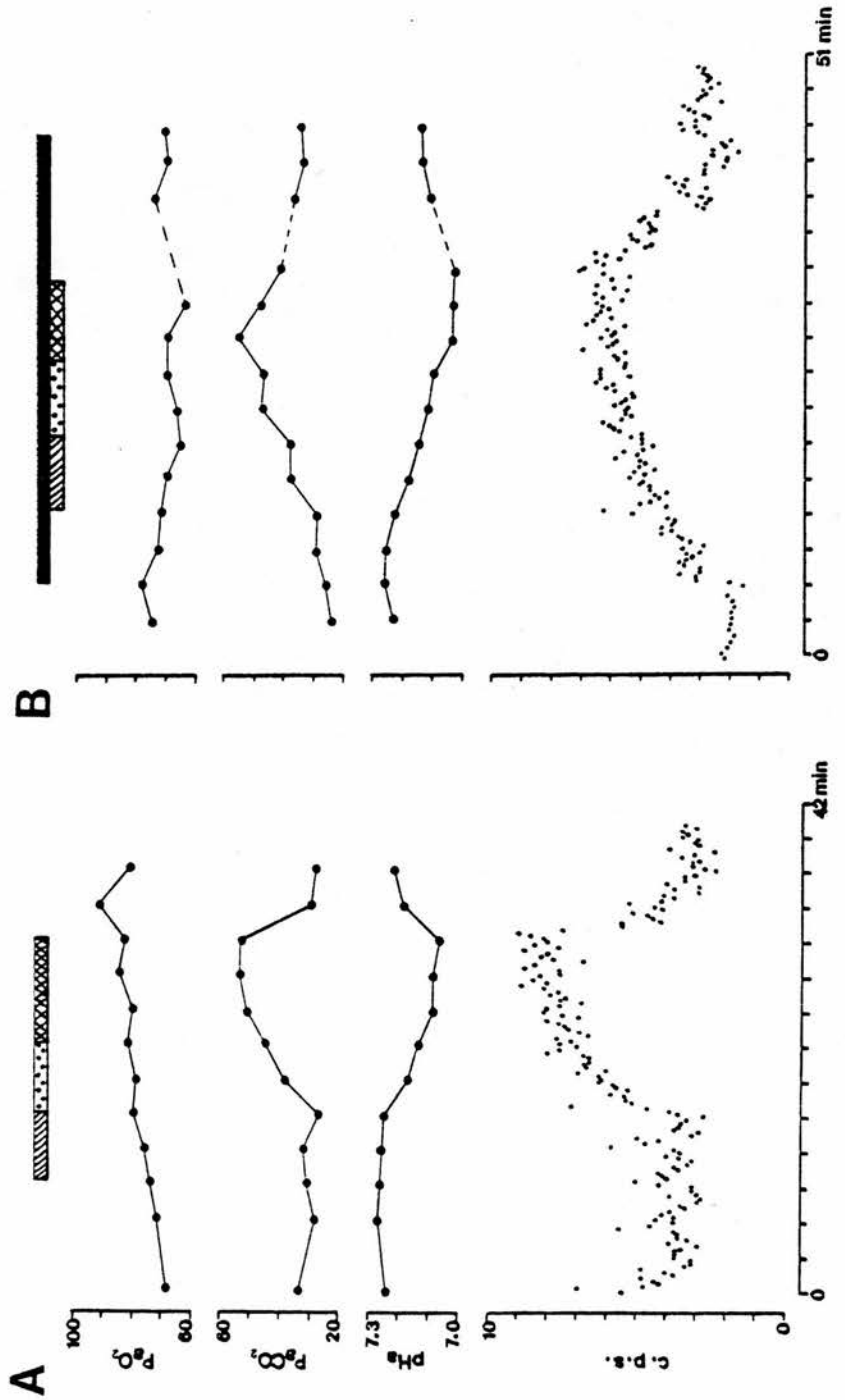

70
P_aO_2
50
60
P_aCO_2
20
7.3
pH_a
7.1
25
c.p.s.
0
0
42
mins

Figure 4.8. (A): Measurements of PaO_2 (mm Hg), $PaCO_2$ (mm Hg), pHa, and discharge frequency ($\bar{x}$ c.p.s. - averaged in consecutive 15 s intervals) recorded in a single experiment, and showing the effects of adding 1% CO_2 (hatched bar), 3% CO_2 (stippled bar) and 5% CO_2 (cross-hatched bar) to the inspired gas.

(B): In this experiment the same parameters were measured during similar changes in inspired gas composition but during the infusion of ISO (10 $\mu g\ min^{-1}$; solid bar).


A
B
100
60
60
20
7.3
7.0
10
0
42 min
51 min

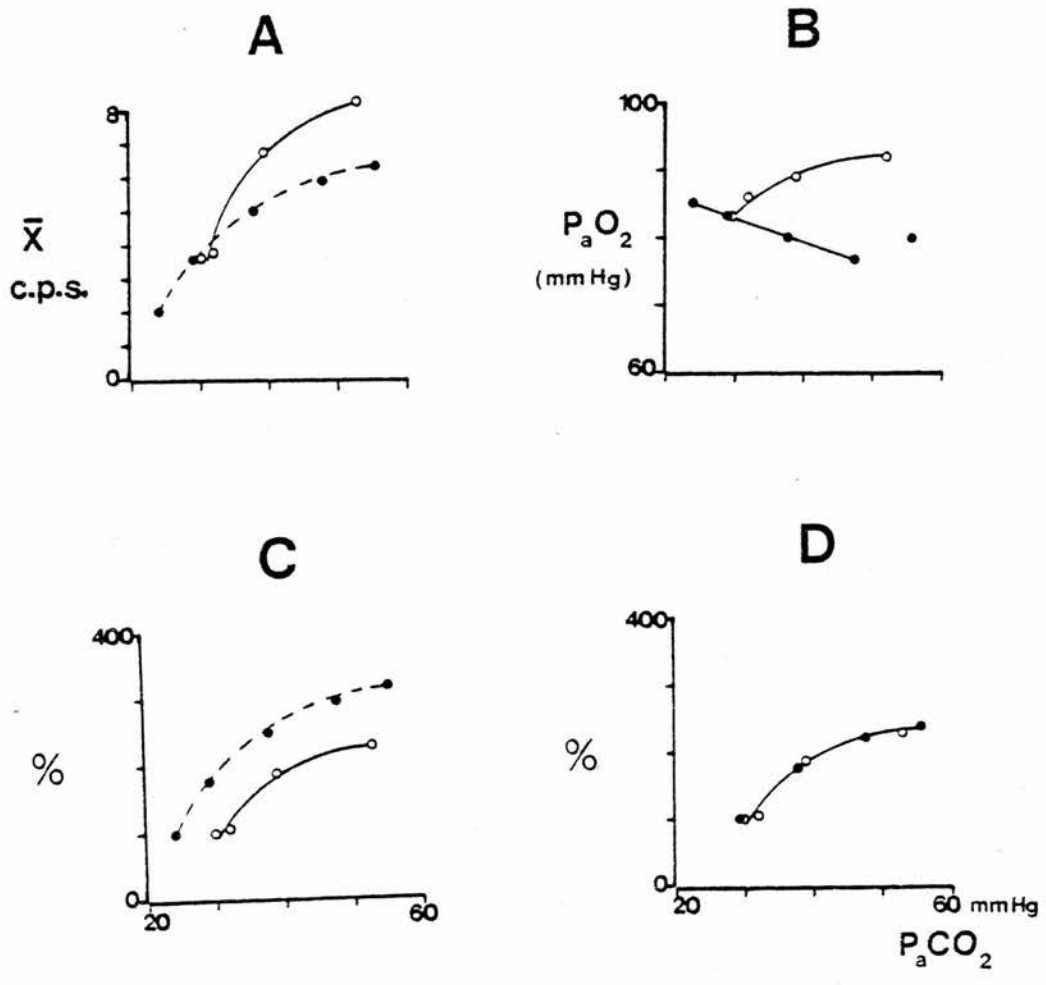


Figure 4.9. Changes in chemoreceptor discharge and PaO_2 in the absence of, and during ISO infusion (10 μg min^{-1}), related to arterial CO_2 tension; this figure illustrates data presented in the previous figure which have been further processed.

A: Average discharge in each 'steady-state' has been plotted as a function of $PaCO_2$ before (O) and during (●) infusion of ISO (10 μg min^{-1}).

B: PaO_2 plotted as a function of $PaCO_2$ before (O) and during (●) infusion of ISO (10 μg min^{-1}).

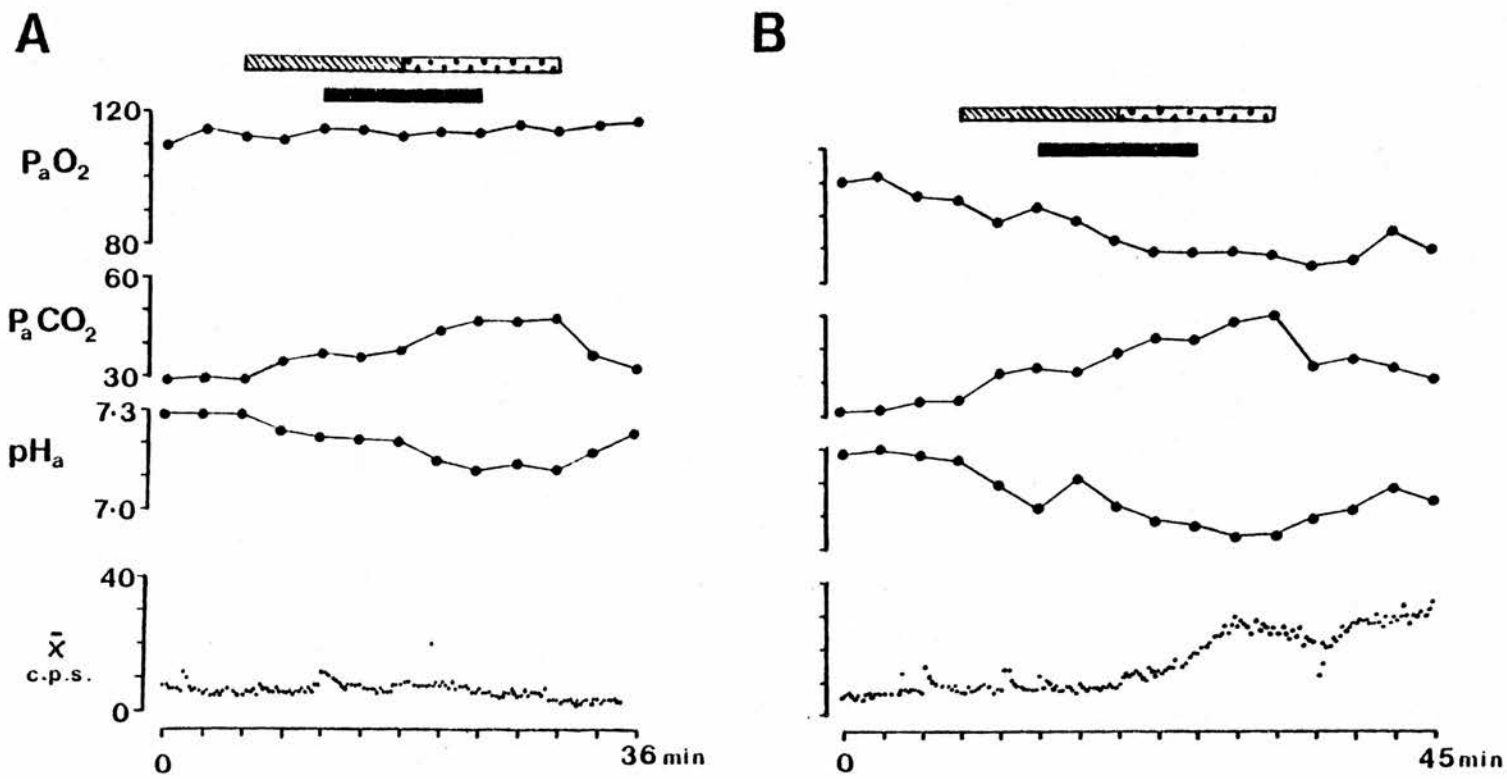
C: Average discharge during each phase of the experiment expressed as a percentage change from the appropriate air-breathing control (100%) plotted as a function of $PaCO_2$ before (O) and during (●) infusion of ISO (10 μg min^{-1}).

D: The data plotted in (C) above have been reworked so as to express the changes elicited by raised CO_2 during ISO infusion as a percentage change from the discharge during ISO infusion whilst breathing air; in this case, there is no obvious difference in the effects of increasing the proportion of CO_2 in the inspired gas.

Lines have been fitted to the data by eye.

Figure 4.10. Data obtained in a single experiment showing (A) effects of the infusion of Locke solution (0.3 ml min^{-1}; solid bar) whilst ventilating with 1% (hatched bar) and 3% (stippled bar) CO_2 added to the inspired gas. PaO_2 values (measurements of PaO_2 and $PaCO_2$ were made in mm Hg) were normoxic throughout the test. Discharge was averaged in consecutive 15 s intervals.

(B): Effects of infusing ISO (10 µg min^{-1}; solid bar) in the same experiment, and under the same conditions.


A
120
P_aO_2
80
60
P_aCO_2
30
7·3
pH_a
7·0
40
$\bar{x}$
c.p.s.
0
0
36 min
B
0
45 min

studied. A striking observation was the suppression by NA (10 μg min^{-1}) of the response to hypercapnia (Fig. 4.11), an effect that became even more noticeable after administration of the β_1-antagonist BET (0.1 mg kg^{-1}; Fig. 4.11). Stopping the infusion of NA during hypercapnia was followed by an instantaneous increase in chemoreceptor activity.

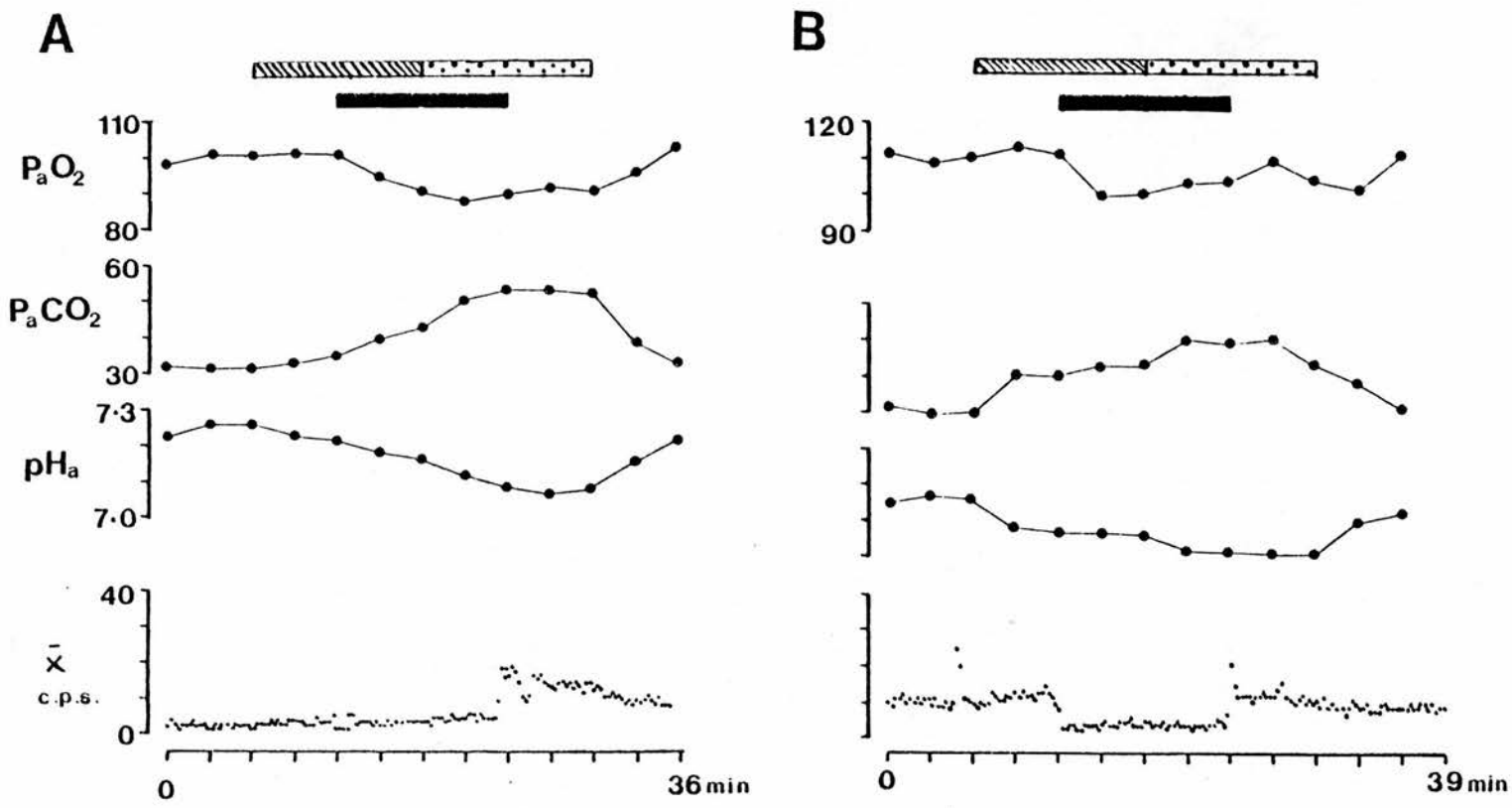
4.3 Interactions of ISO and NA with the 'standard' hypoxia test.

The 'standard' hypoxia test (see Section 2) was carried out during the infusion of NA, ISO, and Locke solution, in three cats. Discharge rate during 10% O_2 in the control experiment (infusion of Locke solution, 0.1 ml min^{-1}) was taken as 100% in each experiment (cf. Section 2), and discharge at the other levels of ventilation, and during infusions was compared with this. Figure 4.12 shows the effects of hypoxia during infusion of Locke solution, and during infusion of ISO at two different rates. Before onset of hypoxia ISO increased discharge in a dose-related manner; 'plateau' discharge during 10% O_2-hypoxia was slightly enhanced by infusion of ISO, but 'peak' discharge during ventilation with nitrogen was not obviously altered. The depression of discharge during ventilation with 100% O_2 was much less effective during infusion of ISO. On returning to air-breathing, during continued ISO infusion, discharge rate was greater than during Locke infusion, but dose-dependency was less marked than in the period before the onset of hypoxia.

In Fig. 4.13 the effects of infusing Locke solution, NA (10 μg min^{-1}), and a very high dose of ISO (100 μg min^{-1}) are compared in the same experiment; at this dose, ISO enhanced chemoreceptor discharge at all stages of the experiment, whilst NA depressed discharge before

Figure 4.11. Data obtained in a single experiment showing (A) effects of the infusion of NA (10 μg min^{-1}; solid bar) whilst ventilating with 1% (hatched bar) and 3% (stippled bar) CO_2 added to the inspired gas. PaO_2 values (measurements of PaO_2 and $PaCO_2$ were made in mm Hg) were generally normoxic. Chemoreceptor discharge was averaged in consecutive 15 s intervals.

(B): Effects of infusing NA (10 μg min^{-1}; solid bar) in the same experiment, and under the same conditions, but following the injection of BET, 0.1 mg kg^{-1}.


A
B
110
80
P_aO_2
60
30
P_aCO_2
7·3
7·0
pH_a
40
0
$\bar{x}$
c.p.s.
0
36 min
120
90
0
39 min

and at the onset of hypoxia, without significantly altering 'plateau' or 'peak' discharge.

Infusion of ISO usually caused a rapid initial increase in chemoreceptor discharge showing an 'overshoot' before rate of discharge stabilised at a new steady-state (cf. Figs 4.12, 4.13, 4.14).

Results of an experiment in which hypoxia tests were performed during catecholamine infusions before and after injecting the β_1-antagonist BET (0.4 mg kg^{-1}) are shown in Fig. 4.14. After the antagonist the depressant effects of NA were enhanced and only a weak response to 10% O_2-hypoxia was obtained. During the infusion of NA or ISO the response to ventilation with 100% N_2 was potentiated, when compared to control curves obtained before the antagonist. The excitatory effect of ISO was clearly not blocked by the β_1-selective antagonist, and the severe hypoxic stimulus of ventilating with 100% N_2 still elicited an increase in chemoreceptor discharge frequency.

In Fig. 4.15 the effects of simultaneous infusion of catecholamines upon the response to hypoxia are demonstrated. ISO infusion (1 and 10 µg min^{-1}; Fig.4.15(1)) slightly reduced chemoreceptor discharge before onset of hypoxia. During 10% O_2-hypoxia the chemoreceptor response was increased in comparison to the Locke-infusion (0.1 ml min^{-1}) control. Infusion of ISO 100 µg min^{-1} caused a pronounced enhancement of discharge at all stages of the test. In a second, separate experiment (Fig.4.15(2)) low levels of ISO infusion (1 and 5 µg min^{-1}) were accompanied by an increase in discharge prior to the onset of hypoxia, whilst the responses to 10% O_2 and 100% N_2 were reduced with respect to control. Infusion of ISO 50 µg min^{-1} was accompanied by an increase in discharge at all stages of the test, except the peak excitatory effect observed during ventilation with

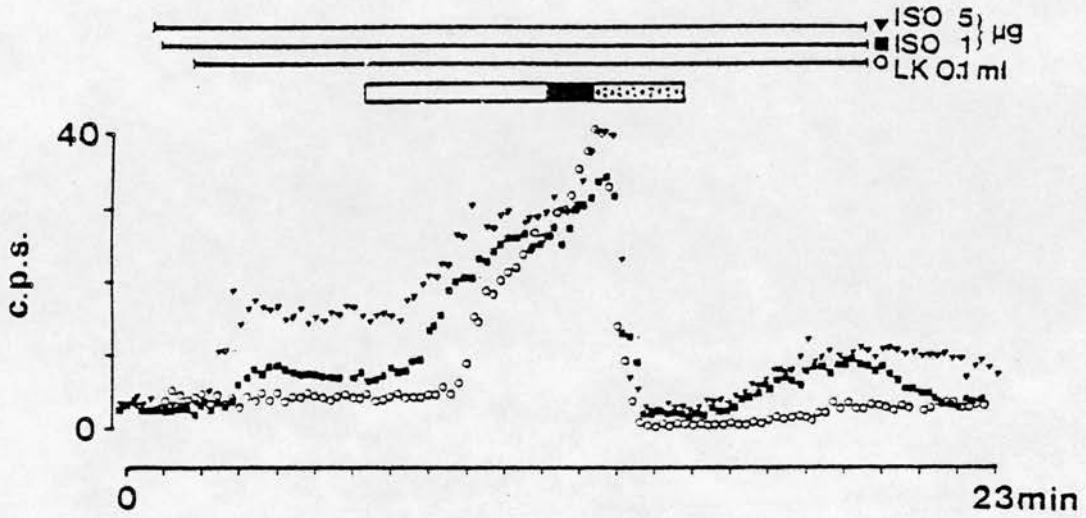


Figure 4.12. Hypoxia tests in a single experiment showing the effects of concomitant infusion of ISO. Discharge, averaged in consecutive 15 s intervals, has been plotted as a function of time. Hypoxia tests (4 min ventilation with 10% O_2 - open bar; 1 min ventilation with 100% N_2 - solid bar; 2 min recovery on 100% O_2 - stippled bar) were performed whilst infusing Locke solution, 0.1 ml min^{-1} (O), and ISO 1 (■) or 5 (▼) µg min^{-1}.

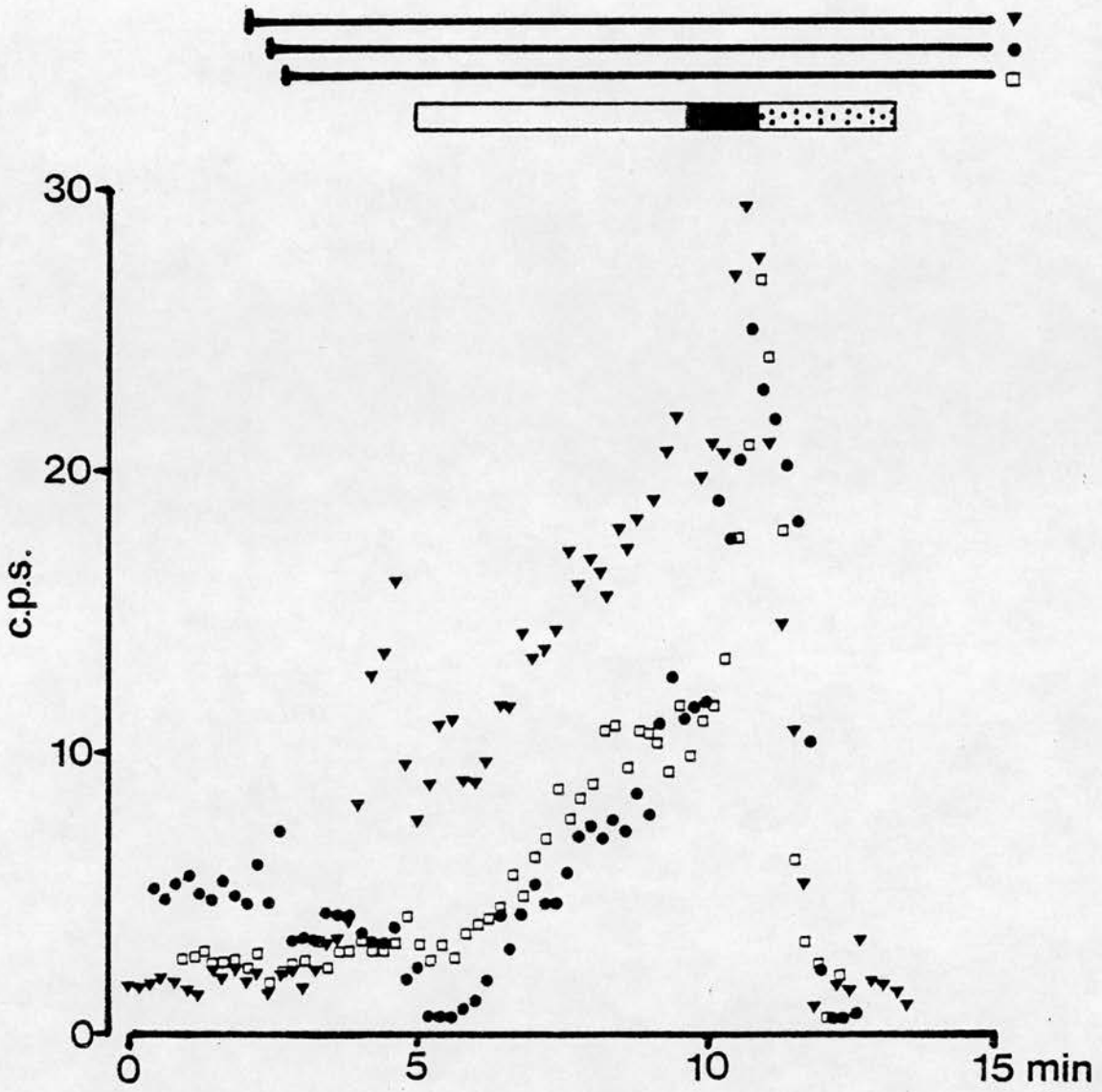


Figure 4.13. Hypoxia tests (4 min ventilation with 10% O_2 - open bar; 1 min ventilation with 100% N_2 - solid bar; 2 min recovery on 100% O_2 - stippled bar) carried out during infusion of (A) Locke solution 0.1 ml min^{-1} (□), (B) ISO 100 µg min^{-1} (▼), and (C) NA 10 µg min^{-1} (●) in a single experiment. Chemoreceptor discharge was averaged in consecutive 15 s intervals. The three sets of data have been superimposed to allow comparison of the differing effects of ISO infusion (which tended to increase the rate of chemoreceptor discharge, except during ventilation with 100% N_2) and NA (which depressed chemoreceptor discharge, before the onset of 10% O_2 hypoxia).

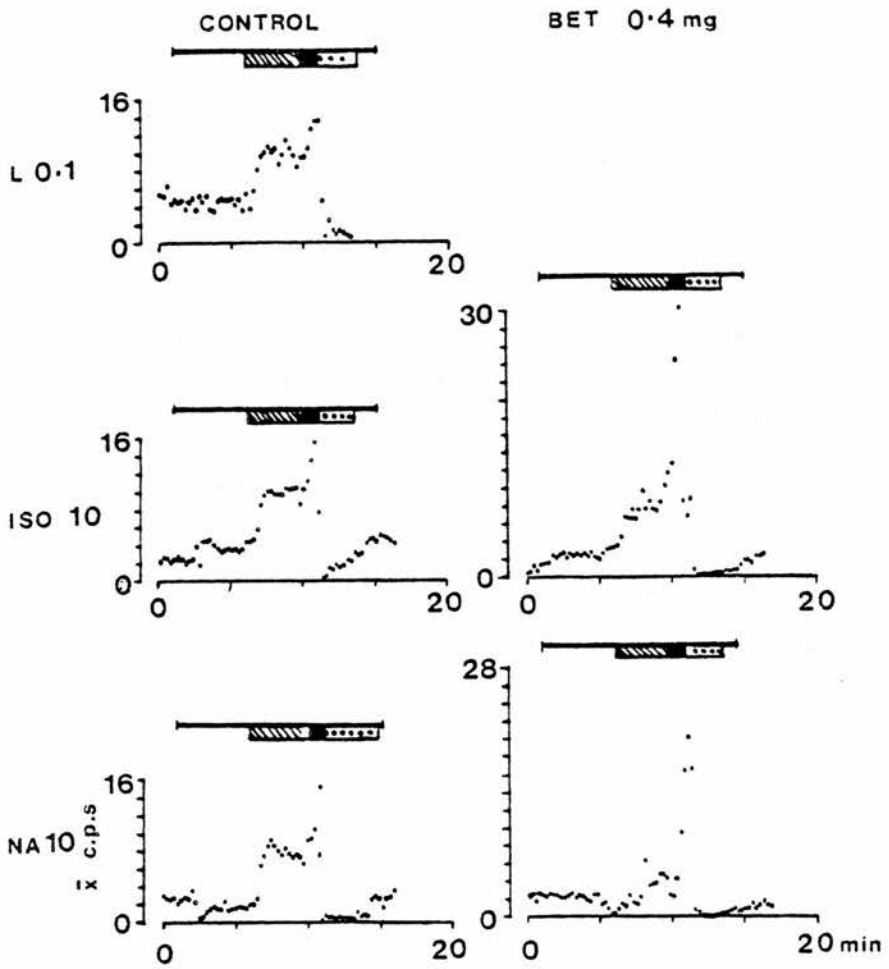


Figure 4.14. Data obtained in a single experiment showing results of hypoxia tests (4 min ventilation with 10% O_2 - open bar; 1 min ventilation with 100% N_2 - solid bar; 2 min recovery on 100% O_2 - stippled bar) carried out during infusion (horizontal bar) of Locke solution 0.1 ml min^{-1}, ISO 10 µg min^{-1}, and NA 10 µg min^{-1}, before (A) and after (B) injecting BET 0.4 mg kg^{-1}. Discharge was averaged in consecutive 15 s intervals.

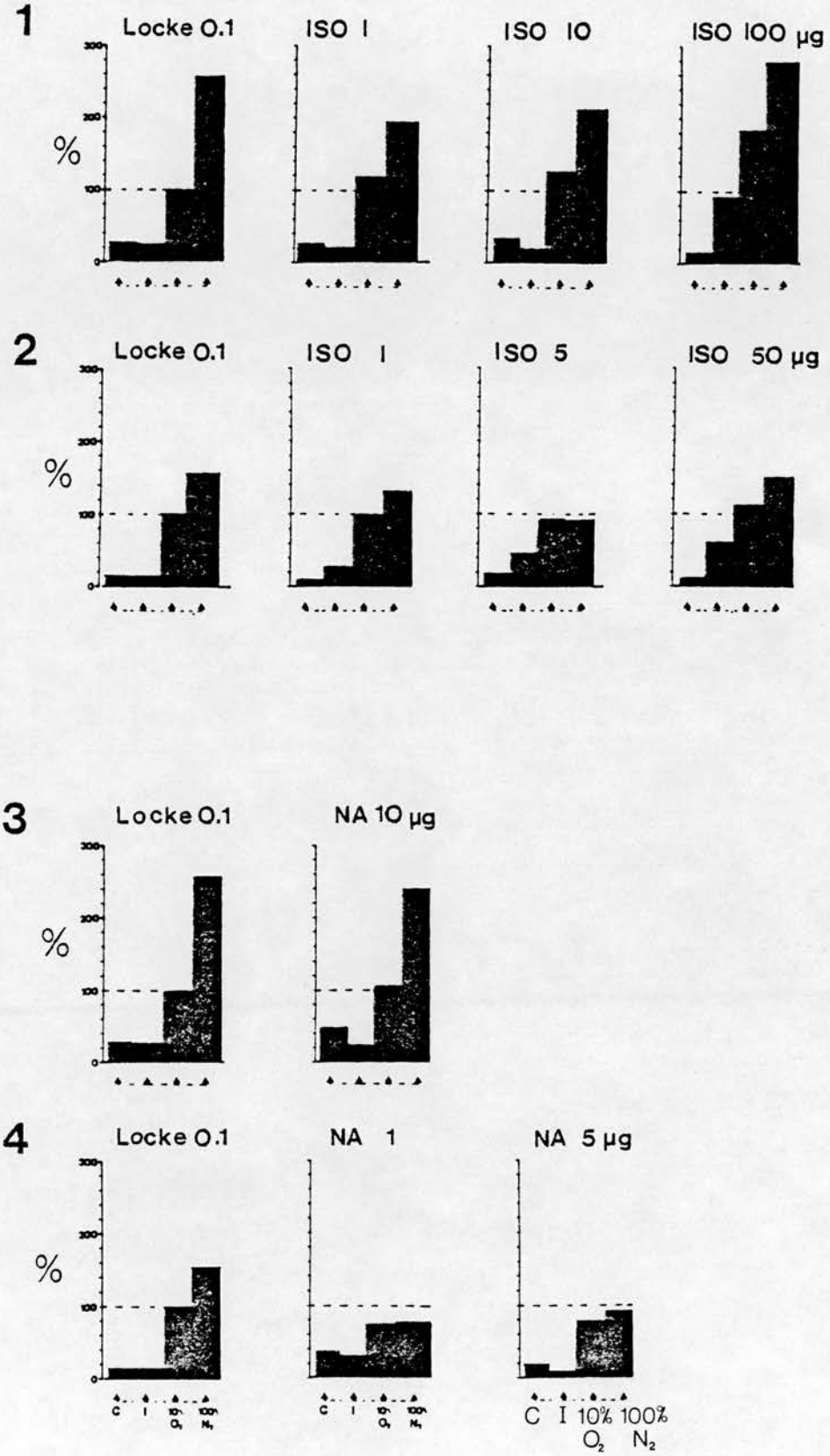
Figure 4.15. Average steady-state discharge during pre-infusion control (C), steady-state during infusion, prior to hypoxia (I), plateau discharge during 10% O_2-hypoxia (10% O_2), and peak discharge whilst ventilating with nitrogen (100% N_2) during infusion of Locke solution (0.1 ml min^{-1}) or ISO (1-100 µg min^{-1}) in a single experiment. To standardise results between individual tests the plateau discharge evoked by 10% O_2-ventilation during Locke infusion was arbitrarily taken as 100% and discharge frequency during the other tests has been related to this level of discharge.

In the first experiment (1), infusion of ISO at the rate of 1 and 10 µg min^{-1} initially decreased chemoreceptor discharge, but there was an increased plateau discharge relative to the Locke infusion control, and a reduced peak discharge during ventilation with 100% N_2. Infusion of ISO 100 µg min^{-1} produced a more marked increase in chemoreceptor discharge.

In the second experiment (2), using the same protocol, ISO (1, 5, and 50 µg min^{-1}) caused a dose related increase in discharge, but no marked changes in the responses elicited by hypoxia.

In the same experiments and under the same conditions, infusion of NA (10 µg min^{-1}) in the first experiment (3) did not markedly alter discharge during the hypoxia test, when compared to Locke control, although infusion of NA caused a reduction in discharge prior to the onset of hypoxia.

In the second experiment (4) infusion of NA 1 and 5 µg min^{-1}, was accompanied by an initial reduction in rate of discharge, and a small reduction in the response of chemoreceptors to hypoxia.


1
Locke 0.1
ISO 1
ISO 10
ISO 100 µg
%
2
Locke 0.1
ISO 1
ISO 5
ISO 50 µg
%
3
Locke 0.1
NA 10 µg
%
4
Locke 0.1
NA 1
NA 5 µg
%
C I 10% 100%
O2 N2

100% N_2.

The corresponding data for tests carried out during infusion of NA in the same experiments show, in both cases, an initial depressant effect of infusing the drug (1-10 μg min^{-1}). In the first experiment (Fig.4.15(3)) the 10% O_2-hypoxia response was marginally potentiated during NA infusion (10 μg min^{-1}) and the peak response evoked by 100% N_2-ventilation was slightly reduced. Both parts of the hypoxia response were reduced during NA infusion (1 or 5 μg min^{-1}) in the second experiment (Fig.4.15(4)).

Figure 4.16 shows results of hypoxia tests performed during infusions of NA before and after cutting the ganglioglomerular nerves. Excitatory responses to high levels of NA infusion (10-50 μg min^{-1}) were more apparent with the sympathetic nerves intact than in any other experiment. After sectioning the sympathetic supply a depressant effect of NA infusion was much more obvious. There was marked variability in the discharge measured in the successive hypoxia tests within the experiment. NA infusion appeared to potentiate the hypoxia response before sympathectomy although there was little correlation with the amount of drug infused. After cutting the sympathetic nerves the response to hypoxia was generally attenuated during infusion of NA.

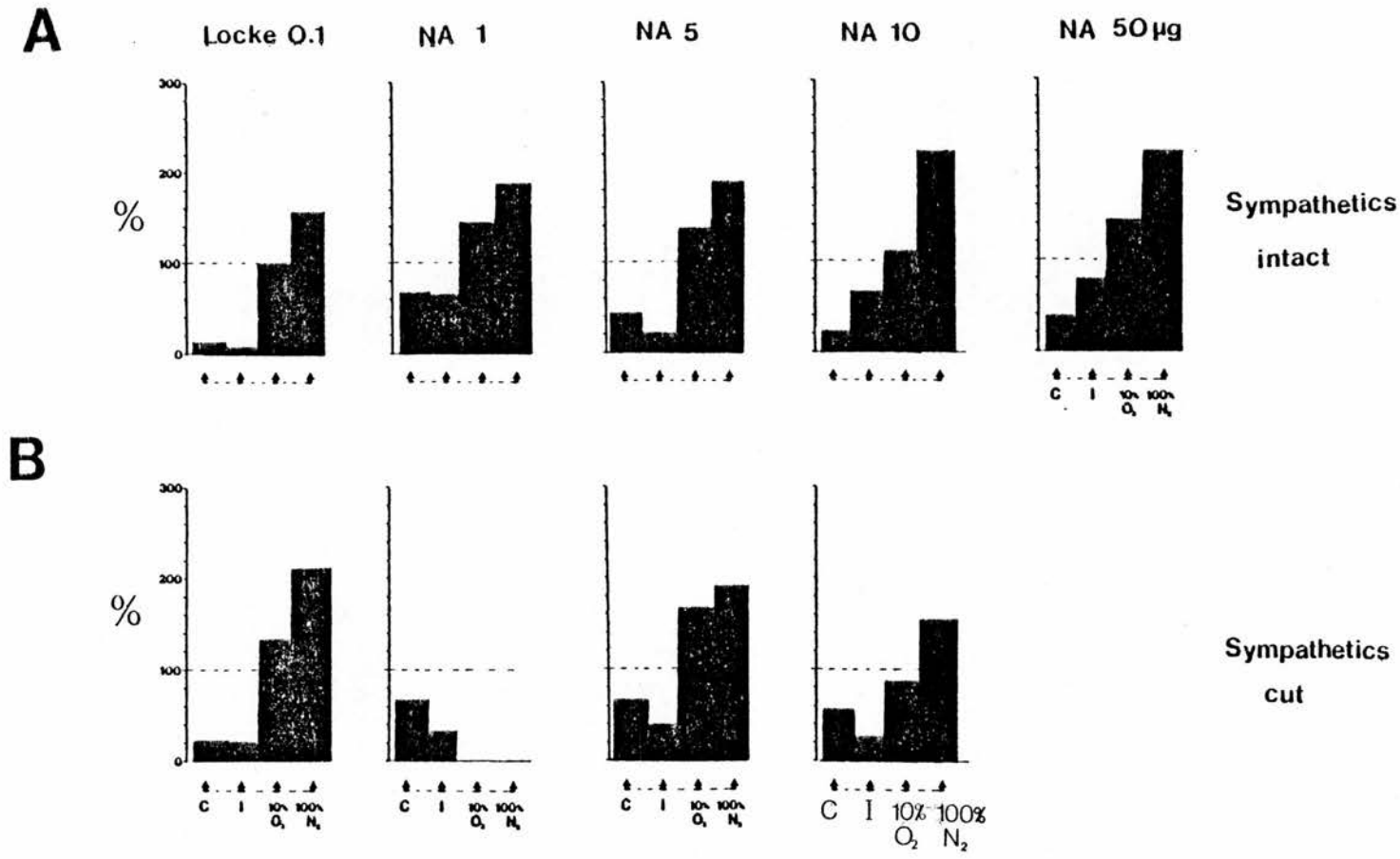
4.4 Effects of β-selective antagonists upon the response of chemoreceptors to hypoxia.

Hypoxia tests were performed before and after administration of the β-selective antagonists PROP (β_1/β_2), MET and BET (β_1), or ICI 118551 (β_2), in order to determine whether the integrity of a β-adrenoceptor mediated mechanism is required for maintainance of the

Figure 4.16. Chemoreceptor discharge during pre-infusion control (C), steady-state during infusion, prior to hypoxia (I), plateau discharge during 10% O_2-hypoxia (10% O_2), and peak discharge whilst ventilating with nitrogen (100% N_2). To standardise results between individual tests the plateau discharge evoked by 10% O_2-ventilation during Locke infusion was arbitrarily taken as 100% and discharge frequency during the other tests has been related to this level of discharge.

In (A), the analysis of hypoxia tests carried out during infusion of Locke (0.1 ml min^{-1}), and NA 1, 5, 10 and 50 µg min^{-1} before sectioning the sympathetic innervation (ganglioglomerular nerves) of the carotid body is shown.

Results presented in (B) were obtained after sectioning the sympathetic nerves. Before neurotomy high rates of NA infusion tended to increase the rate of chemoreceptor discharge, but afterwards a more prominent chemodepressant effect of NA infusion was apparent, though the response to hypoxic stimulation was not markedly reduced.


A
Locke 0.1
NA 1
NA 5
NA 10
NA 50µg
%
300
200
100
0
C
I
10% O2
100% N2
Sympathetics intact
B
%
Sympathetics cut
C I 10% O2 100% N2

chemoreceptor response to hypoxia, as suggested by Folgering et al (1982). In some experiments the effects of α-selective antagonists were also studied.

4.4.1 Results with propranolol (β_1/β_2-antagonist).

Large single doses of PROP (n=4) decreased the basal rate of chemoreceptor discharge and reduced blood pressure and resting heart rate. The responsiveness of chemoreceptors to hypoxia was attenuated, as shown by the reduced level of plateau discharge during ventilation with 10% O_2 (Fig. 4.17). However, the failure of chemoreceptors to respond to hypoxic stimulation was never observed. The degree of hypoxia (PaO_2) attained during ventilation with 10% O_2 was significantly reduced (table 4.3) after PROP.

Discharge on air, plateau discharge during ventilation with 10% O_2, time to plateau, and the rate of increase in discharge in response to hypoxia (slope - cf. Fig. 4.17) were not significantly altered by the antagonist; these parameters are tabulated in table 4.4, which may be compared with Fig. 4.18.

4.4.2 Effects of the β_1-selective antagonist metoprolol.

The effects of MET (n=5) were qualitatively similar to those of PROP, but no marked depression of basal discharge occurred, unlike after PROP. Blood gas analyses performed before and after the antagonist (table 4.5) were not statistically significantly different.

In three of the five experiments there was a reduction in the rate of discharge during air- and 10% O_2- breathing, and in two of these experiments the plateau achieved during ventilation with 10% O_2 was not sustained and discharge continued to decrease even when

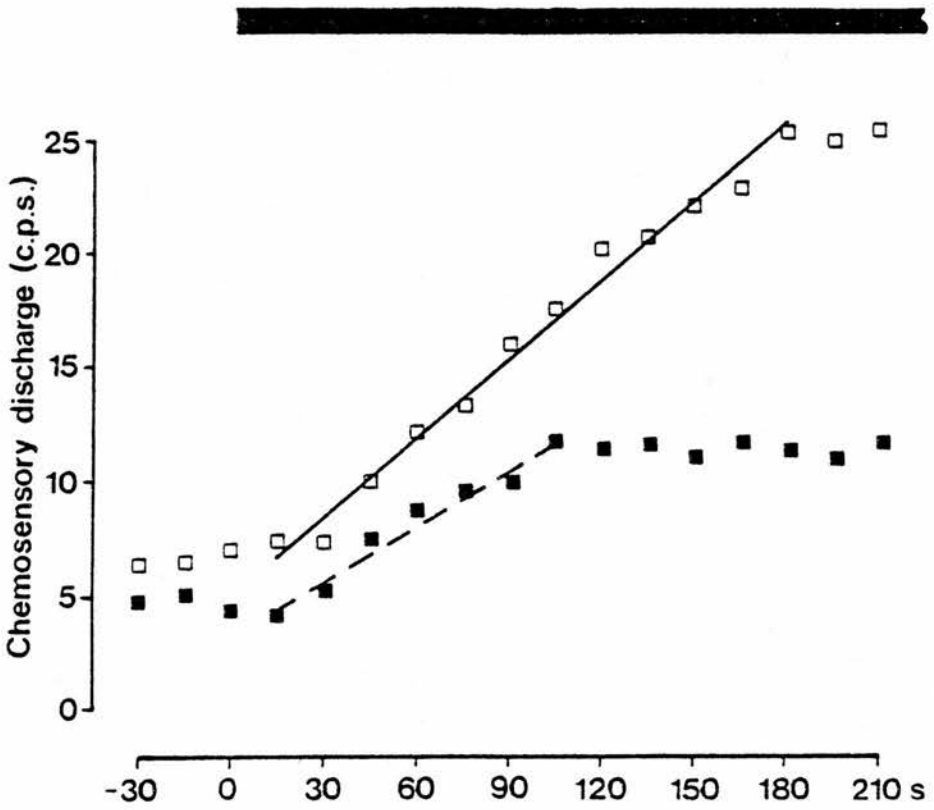


Figure 4.17. Response of chemoreceptors in a single experiment to 10% O_2 hypoxia (solid bar) before (open symbols) and after (closed symbols) injection of PROP (5.8 mg kg^{-1} i.v.). A straight line was fitted to the data in the ranges shown, using the method of least squares.

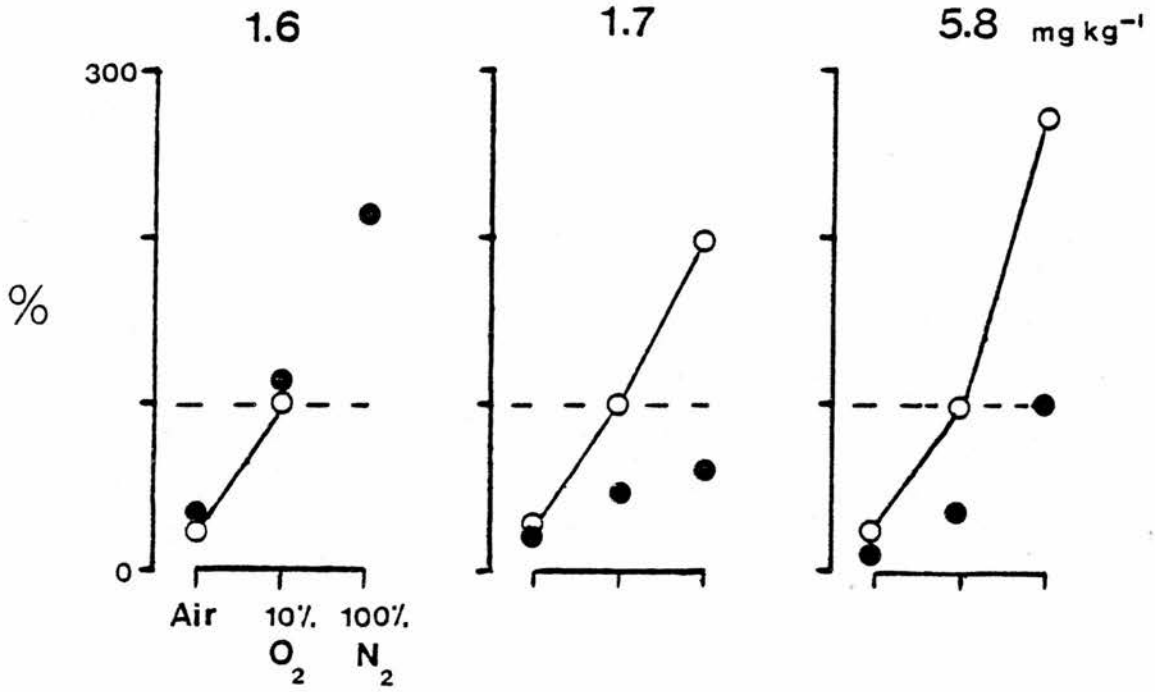


Figure 4.18. Responses of chemoreceptors to hypoxia in three separate experiments before (open symbols) and after (closed symbols) single doses of PROP. Discharge during 10% O_2-hypoxia, before the antagonist, was taken as 100%, and air-breathing discharge (Air) and peak discharge on 100% N_2 were expressed relative to this value, both before and after injecting the antagonist. Doses of PROP used were (left to right) 1.6, 1.7, and 5.8 mg kg^{-1} i.v.

Table 4.3: Results of blood gas analyses carried out before and after propranolol, (A) during air-breathing, and (B) during 10% O_2.

	PaO_2 (mm Hg)		$PaCO_2$ (mm Hg)		pHa	
	Before	After	Before	After	Before	After
(A) [n=2]						
$\bar{x}$	81.0	96.5	33.5	25.0	7.19	7.23
s.e.m.	1.0	3.5	1.5	2.0	0.02	0.03
(B) [n=2]						
$\bar{x}$	41.0	54.0*	29.5	24.0	7.29	7.22
s.e.m.	1.0	0	1.5	2.0	0.02	0.02

*=P<0.05 with respect to pre-drug value (paired t-test)

Table 4.4: Effects of propranolol upon chemoreceptor response to 10% O_2 hypoxia.

	CONTROL	PROPRANOLOL	n
Discharge (air) (c.p.s. ± s.e.m.)	6.7 ± 2.9	3.8 ± 0.8	3
Discharge (10% O_2) (c.p.s. ± s.e.m.)	26.8 ± 12.1	12.1 ± 2.9	3
Discharge (100% N_2) (c.p.s. ± s.e.m.)	68.3 ± 32.8	26.1 ± 11.3	3
Rate of Increase (%max s^{-1} ± s.e.m.)	0.62 ± 0.12	0.36 ± 0.92	3
Time to plateau (s)	148 ± 24	118 ± 21	3

Table 4.5: Results of blood gas analyses carried out before and after metoprolol, (A) during air-breathing, and (B) during 10% O_2.

	PaO_2 (mm Hg)		$PaCO_2$ (mm Hg)		pHa	
	Before	After	Before	After	Before	After
(A) [n=4]						
$\bar{x}$	89.5	97.5	38.0	36.8	7.22	7.14
s.e.m.	7.4	7.3	4.9	6.3	0.07	0.05
(B) [n=2]						
$\bar{x}$	43.0	43.0	28.5	29.5	7.34	7.23
s.e.m.	4.0	1.0	2.5	3.5	0.03	0.09

(No statistically significant differences)

Table 4.6: Effects of metoprolol upon chemoreceptor response to 10% O_2 hypoxia.

	CONTROL	METOPROLOL¶	n
Discharge (air) (c.p.s. ± s.e.m.)	5.1 ± 0.3	5.0 ± 1.2	3
Discharge (10% O_2) (c.p.s. ± s.e.m.)	16.5 ± 1.9	8.7 ± 1.4*	3
Discharge (100% N_2) (c.p.s. ± s.e.m.)	29.0	36.4	1
Rate of Increase (%max s^{-1} ± s.e.m.)	0.68 ± 0.12	0.44 ± 0.06	3
Time to plateau (s)	106 ± 22	84 ± 6	3

	CONTROL	DOMPERIDONE + METOPROLOL†	n
Discharge (air) (c.p.s. ± s.e.m.)	4.1 ± 2.7	5.1 ± 1.6	(4)
Discharge (10% O_2) (c.p.s. ± s.e.m.)	19.6 ± 7.9	21.3 ± 5.8	(4)
Discharge (100% N_2) (c.p.s. ± s.e.m.)	36.7 ± 14.0	33.2 ± 9.5	(4)
Rate of Increase (%max s^{-1} ± s.e.m.)	0.87 ± 0.09	1.05 ± 0.17	(4)
Time to plateau (s)	116 ± 3	110 ± 16	(4)

¶ MET 0.9 mg kg^{-1} (n=1), 2.9 mg kg^{-1} (n=1), & 3 mg kg^{-1} (n=1)
† MET 1 mg kg^{-1} + 3.5 mg kg^{-1} (n=1) & 1 mg kg^{-1} + 1.5 mg kg^{-1} (n=1)
* $P<0.05$ with respect to control (paired t-test).

ventilating with 100% N_2 (Fig. 4.19).

In the other two experiments, where domperidone (100 μg kg^{-1}) had been previously injected, no obvious changes occured (Fig. 4.19), even with relatively high doses of MET.

Statistical analysis of the parameters quantifying the chemoreceptor response to hypoxia before and after MET are shown in table 4.6; discharge during hypoxia, after MET, was significantly lower than control, but this effect was not seen in the presence of domperidone.

4.4.3 Effects of the β_1-selective antagonist betaxolol.

The effects of this antagonist upon heart rate and blood pressure have been described in section 3. There was no marked change in the response of chemoreceptors to 10% O_2-hypoxia after BET in five cats (cf. Figs 4.20 and 4.21), and the discharge elicited by 100% N_2-ventilation was sometimes increased, sometimes reduced (Fig. 4.21). Doses of BET were sufficient to alter resting heart rate and blood pressure and to modify the responses to injected NA, as described in section 3.

Blood gas analyses compared before and after injection of the antagonist (table 4.7) were not significantly different, and comparison of the increased frequency of discharge and rate of increase of discharge in response to hypoxia before and after BET (table 4.8) showed no significant differences. Examination of Fig. 4.22 shows that there were no obvious effects of BET in the presence of previously injected rauwolscine (α_2-antagonist) and ICI 118551.

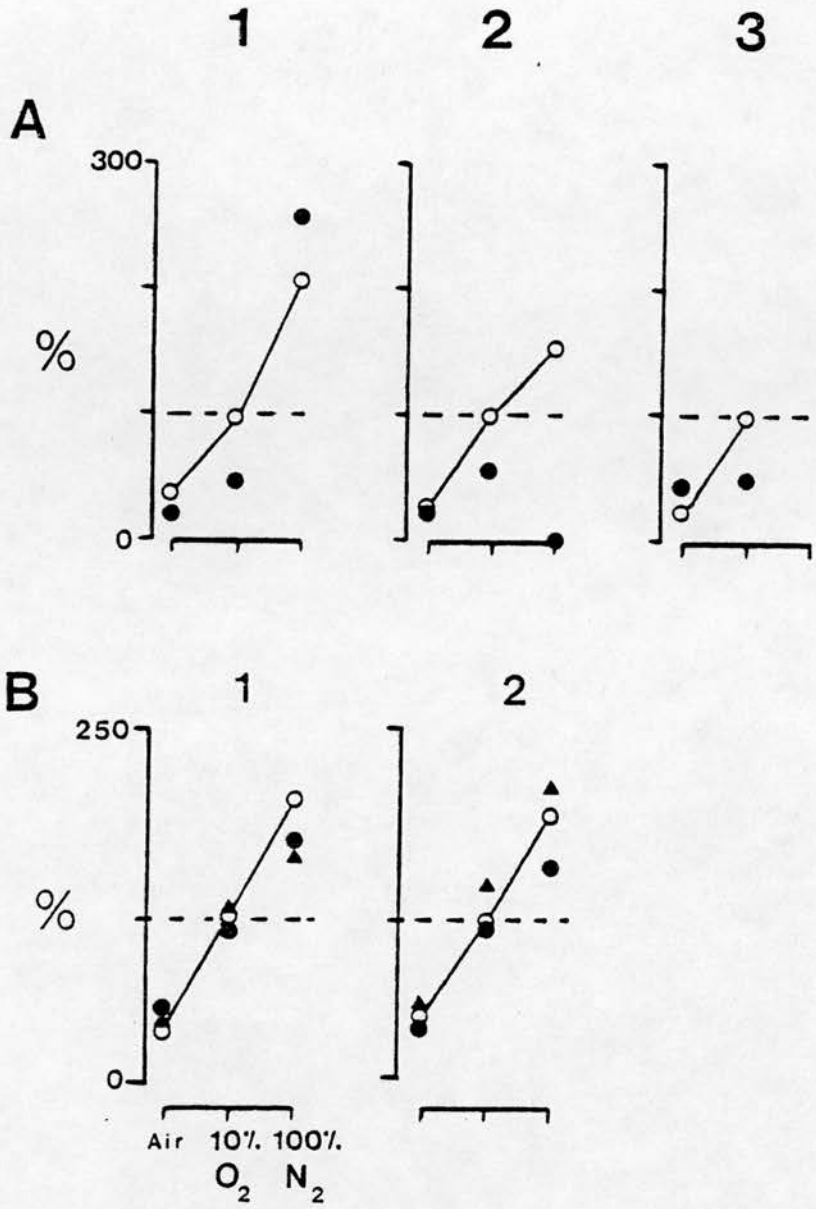


Figure 4.19. (A): Responses of chemoreceptors to hypoxia before (open symbols) and after (closed symbols) MET in three separate experiments. Discharge during 10% O_2-hypoxia, before the antagonist, was taken as 100%, and air-breathing discharge (Air) and peak discharge on 100% N_2 were expressed relative to this value, both before and after injecting the antagonist. Doses of MET used were (left to right) 0.88, 2.9 (twice) and 3.0 mg kg^{-1} i.c.

(B): Effect upon the response to hypoxia of injecting MET 1 mg kg^{-1} (●), and MET 3.5 (▲ - left) or 1.5 (▲ - right) mg kg^{-1} in the presence of domperidone, 100 µg kg^{-1} in two separate experiments. Hypoxia responses before MET but after DP are shown by open symbols.

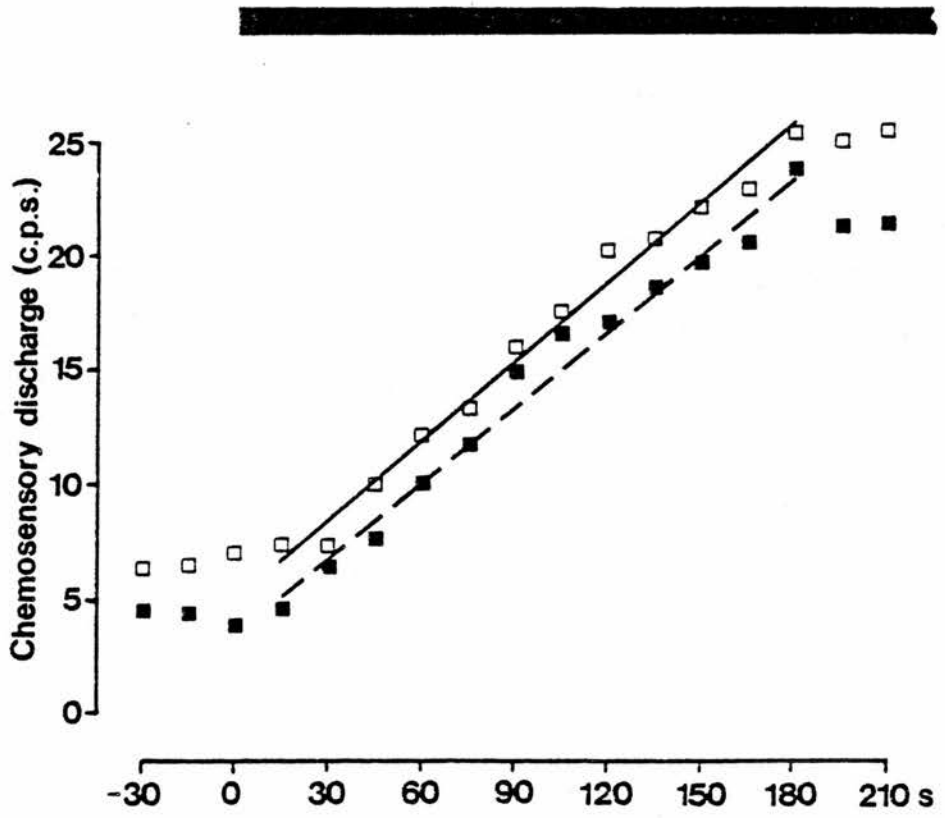


Figure 4.20. Response of chemoreceptors in a single experiment to 10% O_2-hypoxia (solid bar) before (open symbols) and after (closed symbols) injection of BET (1.0 mg kg^{-1} i.c.). A straight line was fitted to the data in the ranges shown, using the method of least squares.

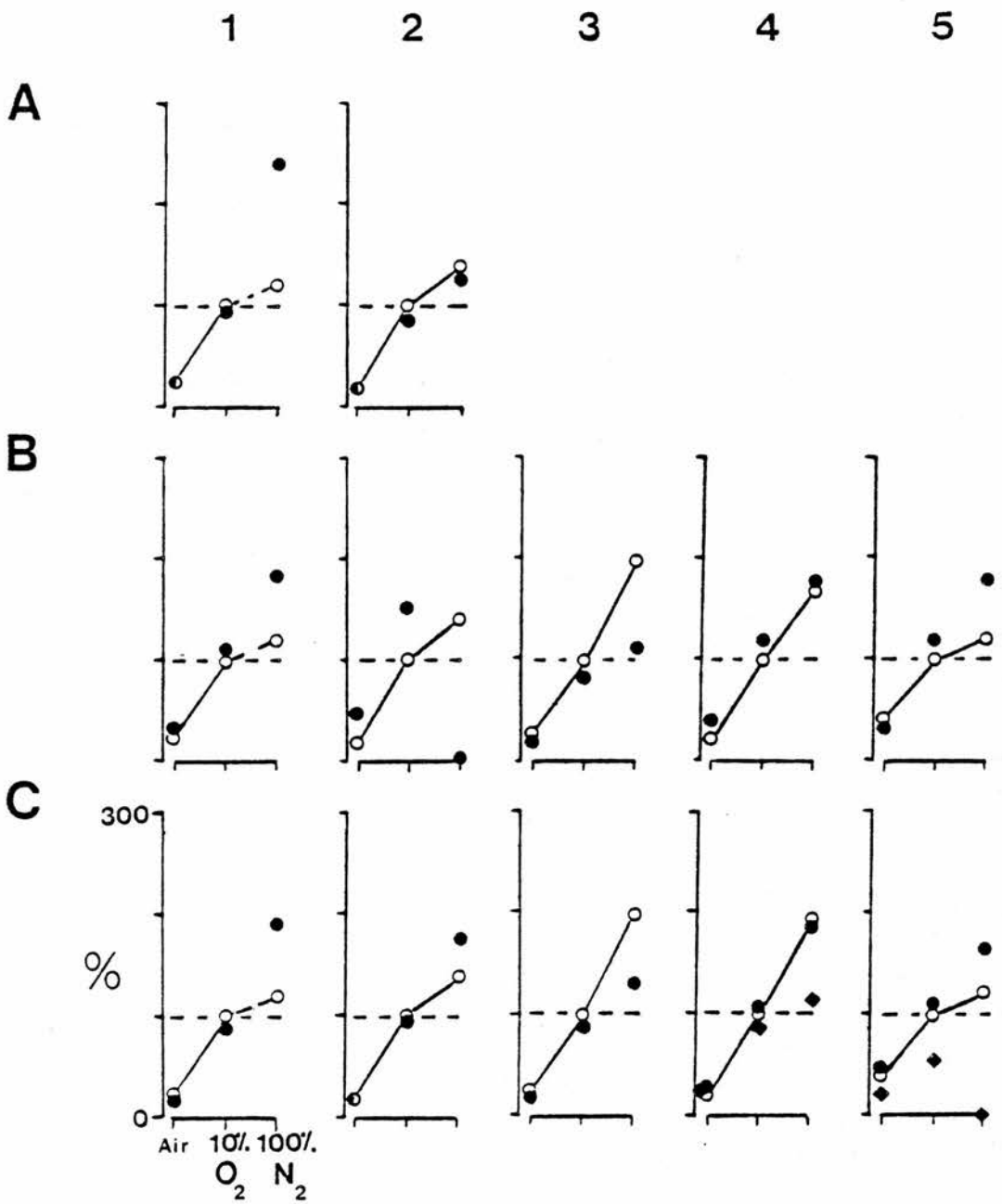


Figure 4.21. Responses of chemoreceptors in five different experiments to hypoxia before (open symbols) and after (closed symbols) BET. Discharge during 10% O_2-hypoxia, before the antagonist, was taken as 100%, and air-breathing discharge (Air) and peak discharge on 100% N_2 were expressed relative to this value, both before and after injecting the antagonist.

Doses of BET used were (A) 10 μg kg^{-1}, (B) 0.1 mg kg^{-1} and (C) 1 mg kg^{-1} i.c. In the last two examples illustrated, data are included for the responses to hypoxia after injecting further doses of BET (◆): 5 mg kg^{-1} (C4) and 2 mg kg^{-1} (C5).

Table 4.7: Results of blood gas analyses carried out before and after betaxolol, (A) during air-breathing, and (B) during 10% O_2.

	PaO_2 (mm Hg)		$PaCO_2$ (mm Hg)		pHa	
	Before	After	Before	After	Before	After
(A)						
100 μg kg^{-1} [n=5]						
$\bar{x}$	102.6	97.6°°	31.0	31.4	7.24	7.24
s.e.m.	10.5	10.2	0.7	0.2	8.4×10^{-3}	0.01
1.0 mg kg^{-1} [n=4]						
$\bar{x}$	88.8	88.8	31.9	29.0	7.24	7.21
s.e.m.	11.2	9.2	0.4	2.1	0.02	0.02
>1.0 mg kg^{-1} [n=2]†						
$\bar{x}$	73.5	79.5	26.5	27.3	7.25	7.27
s.e.m.	9.5	2.5	3.5	4.8	0.03	0.03
(B)						
100 μg kg^{-1} [n=5]						
$\bar{x}$	39.8	42.8	29.1	28.7	7.29	7.28
s.e.m.	4.5	2.5	0.3	0.7	0.01	0.13
1.0 mg kg^{-1} [n=4]						
$\bar{x}$	41.3	40.8	28.6	26.6	7.28	7.30
s.e.m.	2.5	4.3	0.9	1.3	0.02	0.01
>1.0 mg kg^{-1} [n=2]†						
$\bar{x}$	35.0	36.0	24.5	25.5	7.31	7.30
s.e.m.	1.0	1.0	0.5	1.5	0.01	5×10^{-3}

† = 2 mg kg^{-1} (n=1) and 5 mg kg^{-1} (n=1)

°°=P<0.1 with respect to pre-drug value (paired t-test).

Table 4.8: Effects upon chemoreceptor response to 10% O_2 hypoxia of injecting betaxolol (A) 0.01, (B) 0.1, (C) 1, and (D) >1 mg kg^{-1}.

	CONTROL	BETAXOLOL	n
(A) 0.01 mg kg^{-1}			
Discharge (air) (c.p.s. ± s.e.m.)	5.8 ± 4.2	5.2 ± 3.6	2
Discharge (10% O_2) (c.p.s. ± s.e.m.)	29.3 ± 22.7	20.5 ± 3.6	2
Discharge (100% N_2) (c.p.s. ± s.e.m.)	72.0	65.9	1
Rate of Increase (%max s^{-1} ± s.e.m.)	0.66 ± 0.13	0.68 ± 0.11	2
Time to plateau (s)	143 ± 23	112 ± 23	2
(B) 0.1 mg kg^{-1}			
Discharge (air) (c.p.s. ± s.e.m.)	6.9 ± 1.6	11.2 ± 4.5	5
Discharge (10% O_2) (c.p.s. ± s.e.m.)	29.8 ± 9.2	37.2 ± 13.7	5
Discharge (100% N_2) (c.p.s. ± s.e.m.)	55.5 ± 14.2	39.2 ± 16.2	4
Rate of Increase (%max s^{-1} ± s.e.m.)	0.57 ± 0.06	0.54 ± 0.16	5
Time to plateau (s)	153 ± 17	169 ± 17	5
(C) 1 mg kg^{-1}			
Discharge (air) (c.p.s. ± s.e.m.)	6.9 ± 1.6	7.3 ± 2.2	5
Discharge (10% O_2) (c.p.s. ± s.e.m.)	29.8 ± 9.2	29.5 ± 9.5	5
Discharge (100% N_2) (c.p.s. ± s.e.m.)	56.6 ± 14.6	46.8 ± 15.0	4
Rate of Increase (%max s^{-1} ± s.e.m.)	0.57 ± 0.06	0.57 ± 0.06	5
Time to plateau (s)	153 ± 17	162 ± 15	5
(D) >1 mg kg^{-1}†			
Discharge (air) (c.p.s. ± s.e.m.)	8.1 ± 1.9	7.6 ± 4.9	2
Discharge (10% O_2) (c.p.s. ± s.e.m.)	32.6 ± 17.4	26.1 ± 17.9	2
Discharge (100% N_2) (c.p.s. ± s.e.m.)	52.0 ± 33.5	71.0 ± 17.0	2
Rate of Increase (%max s^{-1} ± s.e.m.)	0.55 ± 0.08	0.59 ± 0.05	2
Time to plateau (s)	150 ± 45	102 ± 19	2

† BET 5 mg kg^{-1} (n=1) & 2 mg kg^{-1} (n=1), after the lower doses.

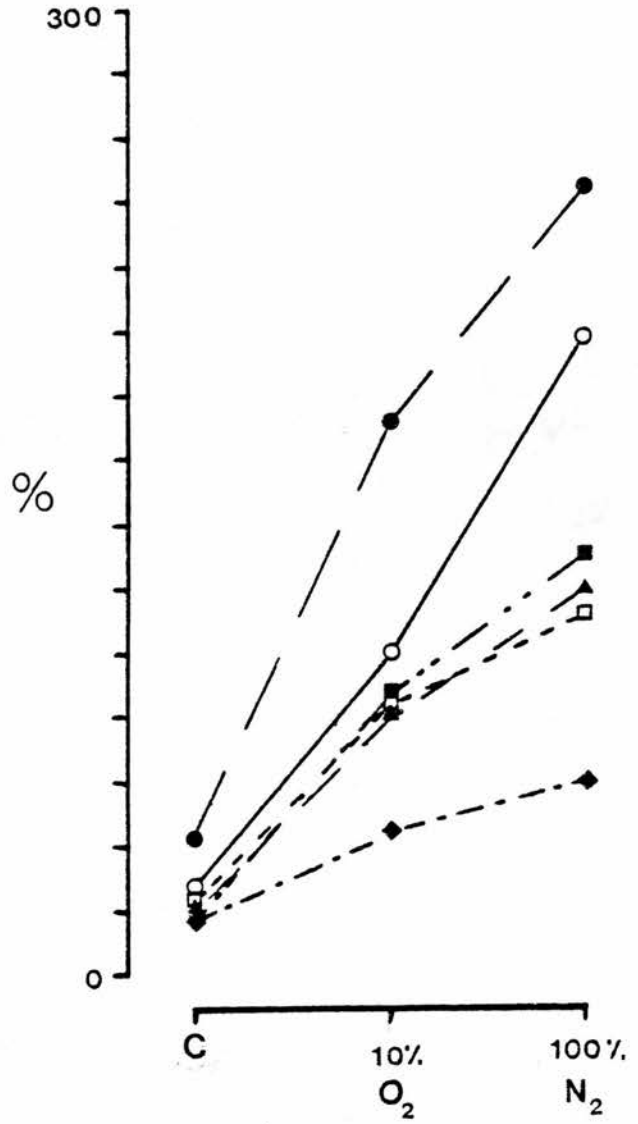


Figure 4.22. Results obtained in a single experiment showing steady-state discharge during air-breathing (C), 10% O_2-hypoxia (10% O_2) and peak discharge during ventilation with 100% nitrogen (100% N_2), before (O), and after

i) ICI 118551 100 µg kg^{-1} (●)
ii) Rauwolscine 1 mg kg^{-1} (▲)
iii) BET 100 µg kg^{-1} (□)
iv) BET 1 mg kg^{-1} (■)
v) PROP 5.8 mg kg^{-1} (◆)

Discharge on 10% O_2 before antagonists has been taken as 100%, and the other levels of discharge related to this.

4.4.4 Effects of the β_2-selective antagonist ICI 118551.

When injected i.c. (0.1-1 mg kg^{-1}; n=2) this antagonist caused a reduction of the hypoxia response in one experiment and a potentiation in the other (Fig. 4.23). In a third experiment, when injected i.v., the antagonist caused no change in the hypoxia response other than a slight reduction of the response to 100% N_2-ventilation (Figs 4.23, 4.24). When ICI 118551 (1 mg kg^{-1} i.c.) was injected after the β_1-selective antagonist BET (6 mg kg^{-1} in cumulative doses) there was a further reduction in the response to hypoxia, already attenuated by the β_1-antagonist (Fig. 4.23). In the remaining experiment, where ICI 118551 was injected i.c., there was a rapid deterioration in the quality of the recording, implying damage to the carotid body. Blood gas analyses performed before and after the antagonist are shown in table 4.9.

Parameters quantifying the response to hypoxia before and after the antagonist are shown in table 4.10. The two sets of data did not differ significantly.

4.5 Summary of results presented in Section 4.

1. Infusions of catecholamine agonists provided qualitatively the same results as injection of these drugs. PHEN (α_1) was inactive, but OXM (α_2) caused chemoexcitation. SAL (β_2) and ISO (β_1/β_2) both caused excitation, and DA depressed chemoreceptor activity. NA caused depression of discharge but an excitatory effect was also observed at higher rates of infusion. OXM effects may have been subject to desensitisation.

2. DP blocked depression of discharge caused by NA infusion, and MET enhanced chemodepression evoked during infusion of NA.

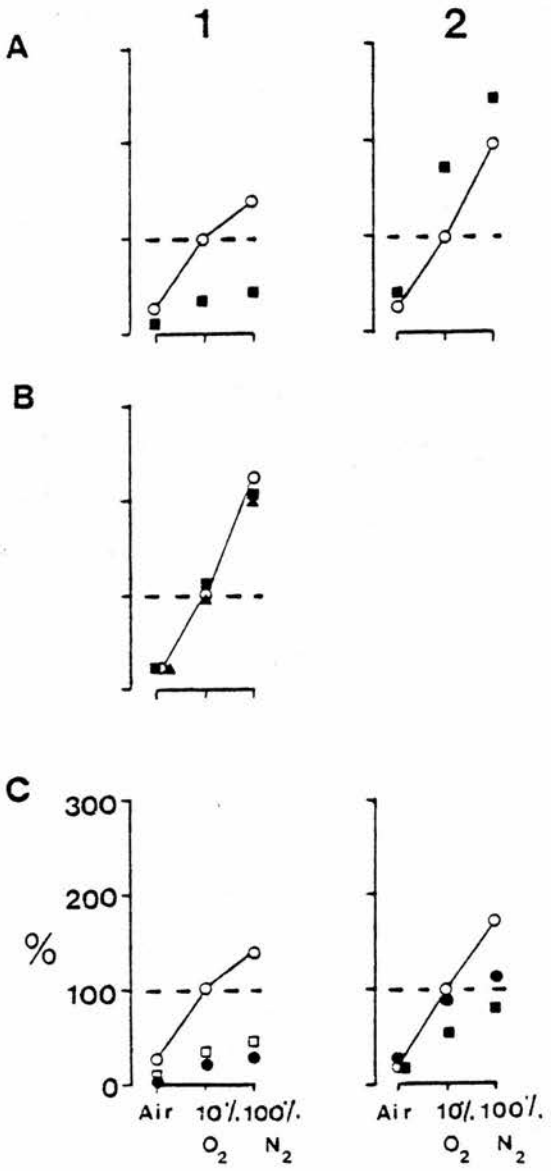


Figure 4.23. (A): Hypoxia responses before (O) and after (■) the injection of ICI 118551 0.1 mg kg^{-1} i.c. in two separate experiments.

(B): Hypoxia responses before (O) and after injecting ICI 118551 0.1 (■) and 1 (▲) mg kg^{-1} i.v. in a single experiment.

(C): Hypoxia responses before (O) and after injecting ICI 118551 1 mg kg^{-1} i.v., (□) followed by BET, 1 mg kg^{-1} i.c. (●) - left, and before (O) and after injecting ICI 118551 (■) 1 mg kg^{-1} i.c., following cumulative doses of BET to a total of 6 mg kg^{-1} (●) - right.

Discharge on 10% O_2 before antagonists has been taken as 100%, and the other levels of discharge related to this.

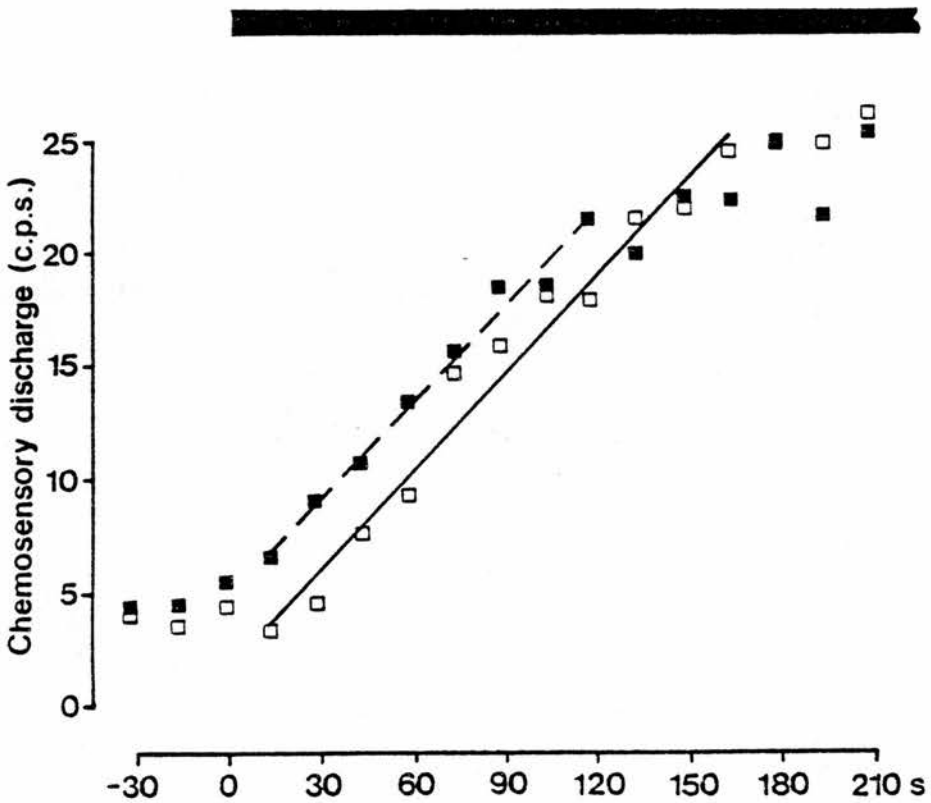


Figure 4.24. Response of chemoreceptors in a single experiment to 10% O_2-hypoxia (solid bar) before (open symbols) and after (closed symbols) injection of ICI 118551 (1 mg kg^{-1} i.v.). A straight line was fitted to the data in the ranges shown, using the method of least squares.

Table 4.9: Results of blood gas analyses carried out before and after ICI 118,551, (A) during air-breathing, and (B) during 10% O_2.

	PaO_2 (mm Hg)		$PaCO_2$ (mm Hg)		pHa	
	Before	After	Before	After	Before	After
(A) [n=5]						
$\bar{x}$	86.4	98.0**	32.8	33.0	7.21	7.23
s.e.m.	9.7	10.0	1.2	1.3	0.02	0.02
(B) [n=5]						
$\bar{x}$	43.2	43.2	30.8	30.4	7.28	7.29
s.e.m.	1.3	2.0	1.6	1.7	0.02	0.03

**=P<0.05 with respect to pre-drug value (paired t-test).

Table 4.10: Effects of ICI 118,551 upon chemoreceptor response to 10% O_2 hypoxia.

	CONTROL	ICI 118551 (100 µg kg^{-1})	n
Discharge (air) (c.p.s. ± s.e.m.)	8.0 ± 2.6	6.9 ± 1.9	3
Discharge (10% O_2) (c.p.s. ± s.e.m.)	32.8 ± 7.8	29.5 ± 13.0	3
Discharge (100% N_2) (c.p.s. ± s.e.m.)	57.7 ± 5.2	44.5 ± 12.1	3
Rate of Increase (%max s^{-1} ± s.e.m.)	0.59 ± 0.08	0.51 ± 0.10	3
Time to plateau (s)	162 ± 26	159 ± 30	3
	CONTROL	ICI 118551 (1.0 mg kg^{-1})	n
Discharge (air) (c.p.s. ± s.e.m.)	7.0 ± 3.0	7.3 ± 2.8	2
Discharge (10% O_2) (c.p.s. ± s.e.m.)	37.4 ± 12.6	25.6 ± 1.5	2
Discharge (100% N_2) (c.p.s. ± s.e.m.)	70.1 ± 15.2	44.2 ± 4.8	2
Rate of Increase (%max s^{-1} ± s.e.m.)	0.52 ± 0.05	0.34 ± 0.13	2
Time to plateau (s)	182 ± 15	176 ± 5	2

3. Infusions of agonists were associated with the same changes in cardiovascular activity as seen after injections of these drugs. Chemoreceptor activity during infusions was occasionally modified by the transient fluctuations in blood pressure caused by the removal of arterial blood samples.

4. ISO increased chemoreceptor discharge concomitant with a reduction in PaO_2. The increase in discharge elicited by increasing inspired CO_2 was additive to the increase in chemoreceptor activity elicited by ISO.

5. Normoxic PaO_2 suppressed the response of chemoreceptors to an increase in inspired CO_2 concentration. NA also suppressed this response (even though it caused a simultaneous fall in PaO_2); this effect was even more marked after the β_1-antagonist BET.

6. ISO infusion enhanced the response to hypoxia in a dose-dependent manner and excited chemoreceptors during ventilation with 100% O_2, but there was no obvious correlation with dose. 'Peak' chemoexcitation during ventilation with 100% N_2 was not obviously enhanced during ISO infusion. NA infusion suppressed chemoreceptor discharge but did not attenuate the response to hypoxia, except after administration of the β_1-antagonist BET. Variability in the chemoreceptor response to hypoxia during infusion did not allow any meaningful quantitative comparison between experiments, or in some cases within a single experiment. Sectioning the ganglioglomerular nerves appeared to potentiate the chemodepressant effects of NA infusion.

7. PROP decreased resting discharge and the maximal discharge rate during 10% O_2-hypoxia; differences were not statistically significant, but the degree of hypoxia (PaO_2) achieved during ventilation with the same hypoxic gas mixture was significantly reduced after PROP. MET

(β_1) reduced the chemoreceptor response to hypoxia, except in the presence of DP, when no change in the response was apparent. BET (β_1) did not attenuate chemoreceptor responses to hypoxia although there was a marked attenuation of cardiovascular responses to NA. The β_2-selective antagonist ICI 118551 sometimes reduced the response of chemoreceptors to hypoxia but could also cause a potentiation of or have no effect upon activity (but damage to the carotid body by close-arterial injection of the drug in an acid vehicle cannot be ruled out).

SECTION 5

STUDIES ON THE EFFECTS OF NORADRENALINE AND ISOPRENALINE IN THE RABBIT

SECTION 5.

STUDIES OF THE EFFECTS OF NORADRENALINE & ISOPRENALINE IN THE RABBIT.

5.1 Introduction.

Compared with the cat and the dog, relatively few studies of the actions of injected neuromodulatory drugs upon chemoreceptor discharge in the rabbit have been presented (cf. Section 1). Since there are indications that the effects of DA and 5-HT upon chemosensory discharge in the cat and the dog are species-specific (Section 1), it is desirable that further studies of the effects of catecholamines and other substances should be carried out in the rabbit. It has already been shown (Docherty & McQueen, 1979) that the effects of injected ACh are quite different in cats and rabbits, the potent chemoexcitatory effects (via nicotinic receptors) in the cat contrasting with the depressant effects (via muscarinic receptors) in the rabbit.

Docherty (1980) studied chemoreceptor activity in 86 rabbits, but obtained only a 30% success rate (compared with a more than 90% success rate in 37 cats). Chemoreceptor afferent activity in the rabbit is conveyed by slowly-conducting, small diameter (<2 μm) myelinated fibres (Laurent & Jäger-Barrès, 1964), which could very easily be damaged during dissection. Chemoafferent fibres in the cat (cf. Section 1) are predominantly fast-conducting A fibres ~3.5 μm diameter - Fidone & Sato, 1969). This low level of success has limited, and continues to limit the amount of information obtained from studies in the rabbit.

Experimental procedures (Section 2) were identical with those used in the experiments on cats.

5.2 Results.

In this study recordings were obtained in nine out of thirteen rabbits (69%), but the quality of recordings was vastly inferior to those obtained in cats (cf. Fig. 5.1); only multiple units were recorded, and almost invariably a large number of baroreceptor action potentials were also recorded, which were often of similar amplitude to the chemoreceptor units.

5.2.1 Dose response data to noradrenaline and isoprenaline.

NA was injected i.c. in five rabbits, over the dose-range 0.01-10 µg (n=3); the range of doses was extended to 100 µg in two rabbits. The chemoreceptor response was qualitatively similar to that seen in cats, although a separate 'E_1'-type excitatory component was not distinguishable.

5.2.1.1 Chemodepression.

In the two experiments where the range of doses was extended to 100 µg, dose-dependent chemodepression was observed in response to NA, with a threshold of about 1 µg (cf. Fig. 5.2).

Domperidone (10 µg kg^{-1}), applied in three experiments, completely blocked NA-evoked chemodepression (e.g. Figs 5.1 and 5.2). In three experiments where the DA D_2-agonist LY 141865* had been injected previously, there was no chemodepression (n=1), slight but dose-related chemodepression (n=1), or chemodepression not obviously related to dose (n=1), following injection of NA.

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* Trans-(±)-4,4a,5,6,7,8,8a,9-octahydro-5-n-propyl-2H-pyrazolo-[3,4-g]-quinoline, dihydrochloride.

Figure 5.1. Neurograms (with ramped counter outputs beneath showing the number of action potentials counted in successive 1 s intervals) of chemoreceptor activity in the rabbit.

A. Effect upon discharge of injecting Locke solution 0.3 ml i.c. (at arrow).

B. Neurogram of chemosensory activity displayed at a faster sweep speed, showing the typical quality of recording in the rabbit; frequency of discharge was high, reflecting the presence of 4-5 units, some of which are of similar amplitude.

C. Effects of injecting DA 1 μg i.c. (at arrow) before and after DP 10 μg kg^{-1}.

D. Effects of injecting NA 10 μg i.c. (at arrow) and E, NA 50 μg i.c. (at arrow) before and after DP 10 μg kg^{-1},

F. Effects of injecting ISO 10 μg i.c. (at arrow) before and after DP 10 μg kg^{-1}.

Note the increase in basal discharge frequency after the antagonist.

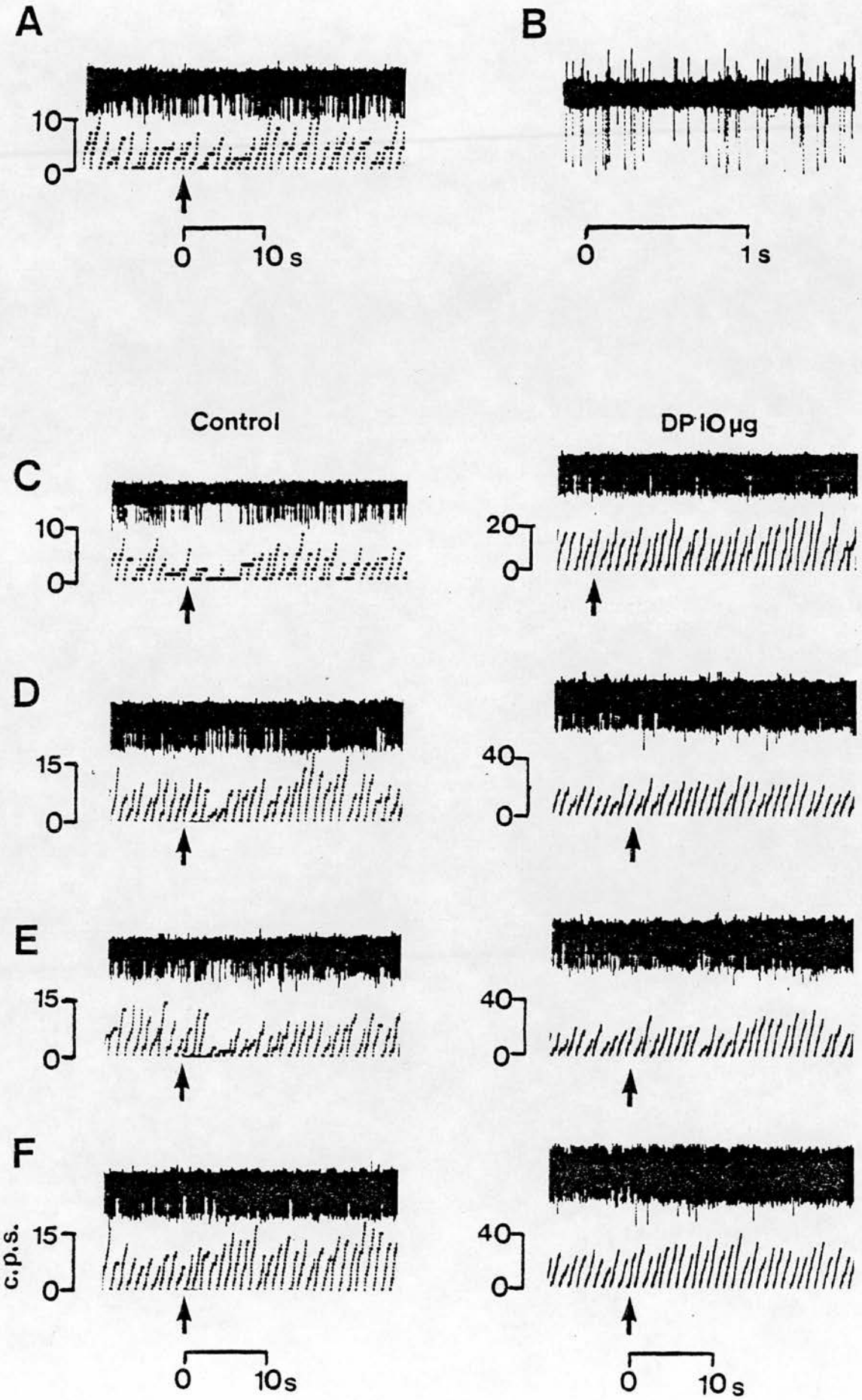
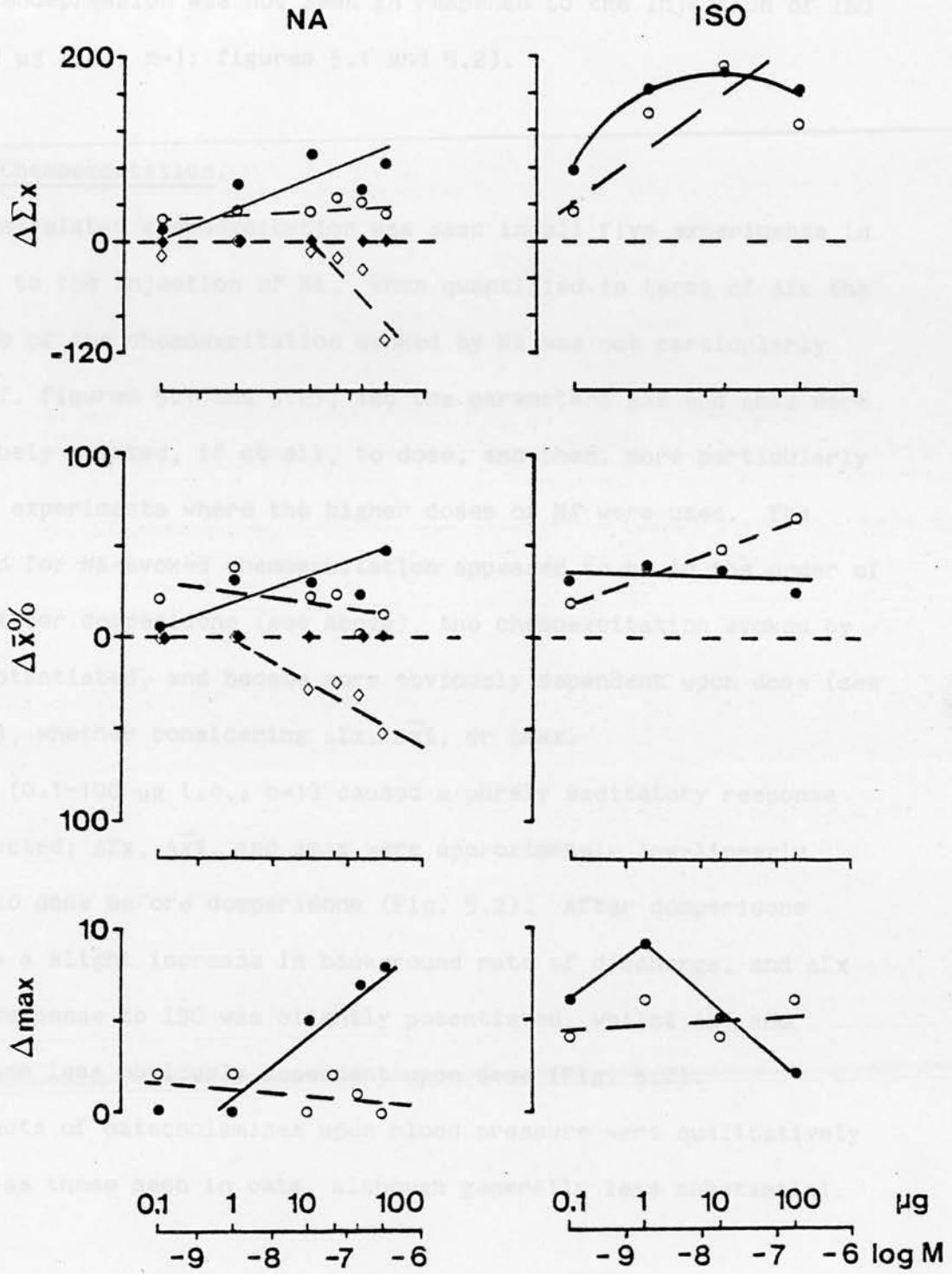

A
10
0
0
10 s
B
0
1 s
Control
DP 10 µg
C
10
0
20
0
D
15
0
40
0
E
15
0
40
0
F
c.p.s.
15
0
40
0
0
10s
0
10 s

Figure 5.2. Dose-response data for the effects upon chemoreceptor discharge of injections of NA (0.1-100 μg i.c.) and ISO (0.1-100), before (open symbols) and after (closed symbols) DP 10 μg kg^{-1}.

Dose-related chemodepression (◇) was evoked by doses of NA > 10 μg, and this effect was abolished by DP. Only slight, non-dose-dependent chemoexcitation (○) followed chemodepression, but became greater and more dose-dependent after DP (●).

ISO did not cause chemodepression, but did elicit dose-dependent chemoexcitation before DP; Δmax was less clearly related to dose after the antagonist, although the chemoexcitatory effect ($\Delta\Sigma x$) was not obviously altered.

Data are presented as integrated responses ($\Delta\Sigma x$ counts), $\Delta\bar{x}\%$, and Δmax.


NA
ISO
200
0
-120
ΔΣx
100
0
100
Δx̄%
10
0
Δmax
0.1
1
10
100
µg
-9
-8
-7
-6
log M

Chemodepression was not seen in response to the injection of ISO (0.1-100 μg i.c., n=1; figures 5.1 and 5.2).

5.2.1.2 Chemoexcitation.

Dose-related chemoexcitation was seen in all five experiments in response to the injection of NA. When quantified in terms of $\Delta\Sigma x$ the magnitude of the chemoexcitation evoked by NA was not particularly great (cf. figures 5.1 and 5.2), and the parameters $\Delta\bar{x}\%$ and Δmax were only vaguely related, if at all, to dose, and then, more particularly in those experiments where the higher doses of NA were used. The threshold for NA-evoked chemoexcitation appeared to be in the order of 10 μg. After domperidone (see above), the chemoexcitation evoked by NA was potentiated, and became more obviously dependent upon dose (see Fig. 5.2), whether considering $\Delta\Sigma x$, $\Delta\bar{x}\%$, or Δmax.

ISO (0.1-100 μg i.c., n=1) caused a purely excitatory response when injected; $\Delta\Sigma x$, $\Delta\bar{x}\%$, and Δmax were approximately log-linearly related to dose before domperidone (Fig. 5.2). After domperidone there was a slight increase in background rate of discharge, and $\Delta\Sigma x$ for the response to ISO was slightly potentiated, whilst $\Delta\bar{x}\%$ and Δmax became less obviously dependent upon dose (Fig. 5.2).

Effects of catecholamines upon blood pressure were qualitatively the same as those seen in cats, although generally less substantial.

5.2.2 Studies of the effects of infusions of noradrenaline and isoprenaline, and their interactions with the response to hypoxia.

5.2.2.1 Infusions of noradrenaline.

NA (1-50 μg min^{-1}) was infused in five rabbits, in all but one of

which LY 141865 had been previously injected in an independent experiment. Results fell broadly into two categories: those in which the effect of the amine was excitatory, and those in which it depressed chemoreceptor discharge (see table 5.1).

The experiment illustrated in Fig. 5.3 shows an apparent dose-dependency of the net effect upon chemoreceptor discharge of infusing NA; discharge in the steady-state correlated well with $\log_{10}$ dose (Fig. 5.4A), suggesting that chemodepression predominated when low amounts of the drug were infused, with the excitatory component becoming more apparent as the amount of NA infusion per unit time was increased.

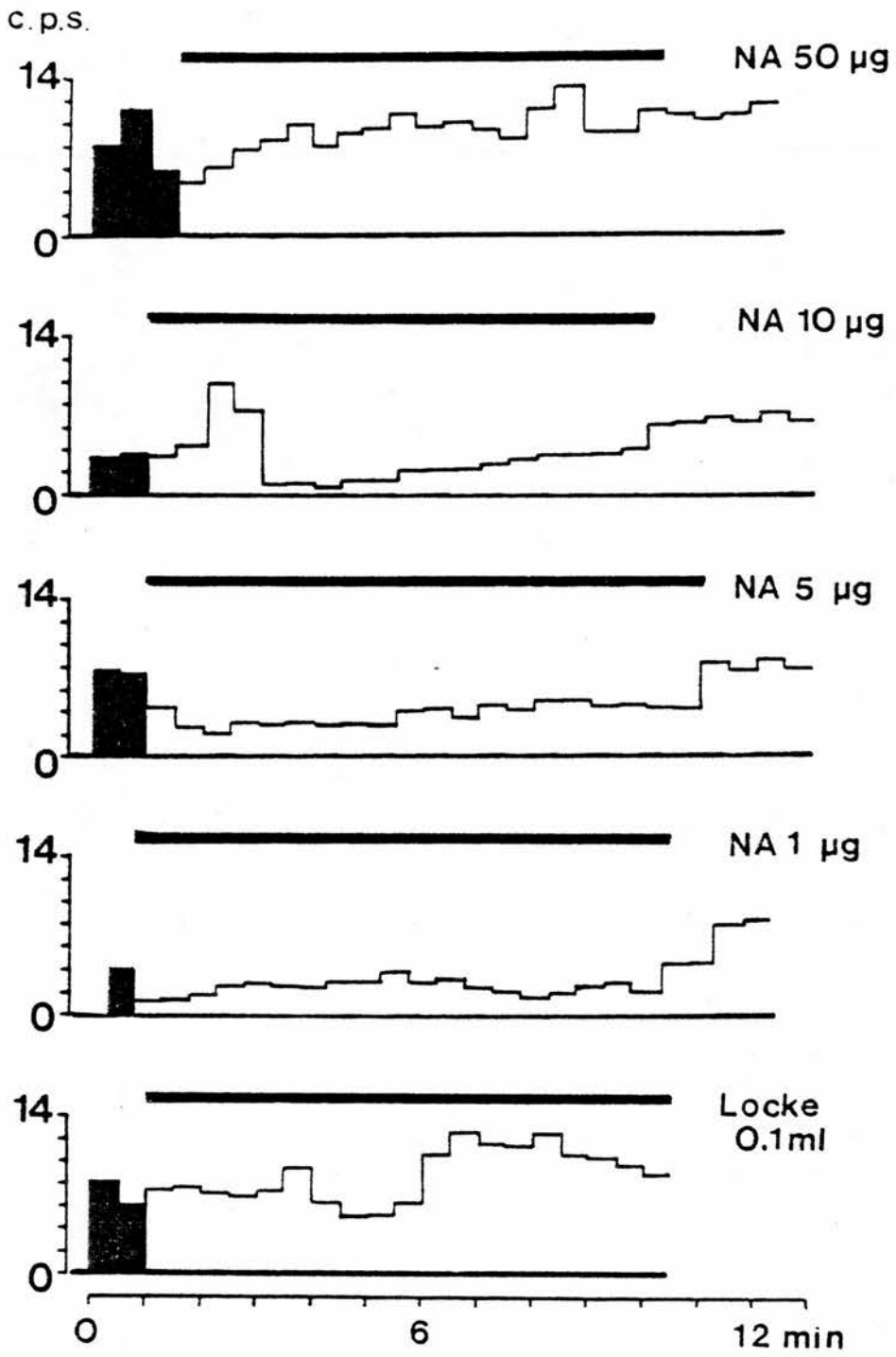
In the presence of the highest doses of LY 141865, only excitatory responses to infusion of NA were seen (the compound may act as a partial agonist - McQueen et al, 1984). Where tested, chemodepressant effects of NA infusion were 'converted' to excitatory responses after injection of domperidone (10 μg kg^{-1}, n=2 - although in one of the experiments (number 3 in table 5.2) excitatory responses to infusions of low doses of NA were not well sustained, and in response to a high dose of NA, no change in discharge was seen), or L-sulpiride (100 μg kg^{-1}, n=1).

One experiment (number 4 in table 5.2) was anomalous in so far as no steady-state was apparent: soon after onset of infusion there was a rapid rise in chemoreceptor discharge, which then declined to a near constant level of firing which was variously greater than, or less than in the pre-injection control. Domperidone (two doses of 10 μg kg^{-1}) did not alter this pattern of response, whilst the dextro-rotatory isomer of propranolol (10 mg kg^{-1}) caused a prolongation of the 'peak' increase in discharge during infusion of NA (50 μg min^{-1}), and after injecting the racemic preparation of propranolol (1.4 mg kg^{-1},

Table 5.1. Mean steady-state discharge ($\bar{x}$ c.p.s.) during air-breathing before and after onset of infusion of NA or ISO, discharge during 10% O_2-hypoxia, and peak discharge during ventilation with 100 % N_2 in a rabbit. Data are tabulated in the order in which infusions were carried out.

No.	Infusion (ml or µg min^{-1})	Control (air)	Infusion (air)	Infusion +10% O_2	Infusion +100% N_2
1	ISO 10	12.5	16.0	28.0	54.0
2	Locke 0.1	1.9	1.3	5.6	8.5
3	ISO 1	2.7	3.0	8.0	10.1
4	ISO 50	3.0	5.5	10.5	25.0
5	NA 10	2.8	1.4	9.0	15.7
6	NA 50	2.6	0.6	7.1	11.3
7	Locke 0.1	1.6	2.0	7.7	14.2
After MET 1 mg kg^{-1}					
8	Locke 0.1	1.5	1.4	4.8	10.9
9	ISO 50	4.0	5.0	15.0	23.0
10	NA 50	11.0	2.5	19.0	30.0
After L-sulpiride 100 µg kg^{-1}					
11	NA 50	2.1	3.3	5.3	6.7
12	NA 50	0.6	1.2	6.4	12.4
After PROP 1.4 mg kg^{-1}					
13	ISO 50	5.4	6.0	9.4	11.4

Figure 5.3. Responses of chemoreceptors to infusions (solid bar) of Locke solution (0.1 ml min^{-1}) and NA (1-50 $\mu g\ min^{-1}$). Discharge was averaged in successive 30 s intervals, and plotted against time. The lower concentrations of NA infused caused a reduction in discharge frequency from pre-infusion control levels (solid blocks); infusion of NA 10 $\mu g\ min^{-1}$ resulted in an initial excitation, which gave way to a pronounced, if poorly-sustained depression of discharge. Infusion of NA 50 $\mu g\ min^{-1}$ was associated with only an excitatory effect.


c.p.s.
14
0
NA 50 µg
NA 10 µg
NA 5 µg
NA 1 µg
Locke
0.1ml
0
6
12 min

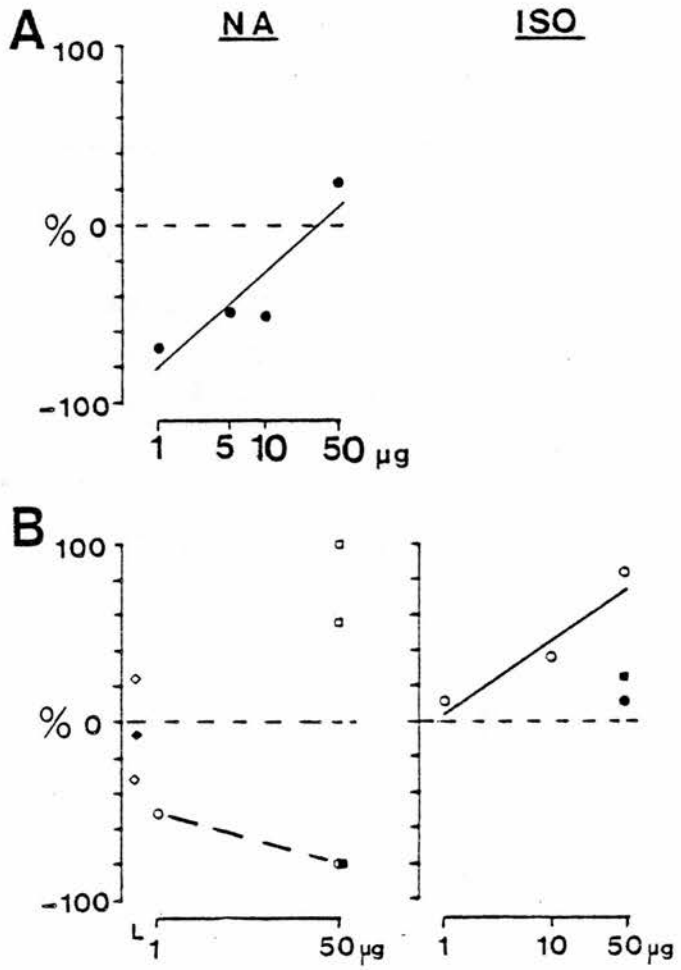


Figure 5.4. A. From the same experiment as shown in Fig. 5.3 average discharge in the steady-state achieved during infusion of NA has been expressed as a percentage change from the average rate of discharge in the respective pre-infusion control, and plotted against the amount of NA infused (µg min^{-1}) on a log_{10} scale. A straight line was fitted to the data points by the method of least squares.

B. Infusion data from a separate experiment treated as above (A). Infusion of NA (○) 1 or 50 µg min^{-1} caused a dose-related depression of discharge. After MET (3.5 mg kg^{-1}) the chemodepressant effect of infusing NA at the higher rate was unaltered (■). Two infusions of NA at the higher concentration caused increases of ~50% and nearly 100% above pre-infusion control discharge after L-sulpiride 100 µg kg^{-1} (□). Also shown are the steady-state effects of infusing Locke solution (0.1 ml min^{-1}). Before antagonists, Locke-infusion (◇) caused either a slight depression of discharge (cf. Fig. 5.5 1A), or, during a separate infusion, a slight increase in chemoreceptor discharge. After MET (◆) the same infusion caused a much smaller depression of discharge (cf. Fig. 5.5 1B).

Infusion of ISO (1-50 µg min^{-1}) in this experiment caused a dose-related increase in discharge, at steady-state. After MET (3.5 mg kg^{-1}) the increase in discharge elicited by infusion of ISO (50 µg min^{-1}; ■) was greatly attenuated, and even more so after administration of L-sulpiride (100 µg kg^{-1}) and PROP (1.4 mg kg^{-1}; ●).

Table 5.2. Steady-state discharge during NA infusion, expressed as a percentage change from pre-infusion discharge, before and after domperidone, and, in some experiments, in the presence of LY 141865.

			CONTROL		After DOMPERIDONE*	
Expt. No.	Infusion rate (NA μg min^{-1})	LY † (μg kg^{-1})	Depression (%)	Excitation (%)	Depression (%)	Excitation (%)
1	1	3.0	-68	-		
	5		-49	-	(NOT	
	10		-50	-	TESTED)	
	50		-	+25		
2	10	16.0	-58	-	-	+33
	50		-	+338	-	+60
3	10	29.0	-	+10	-	+29→-10
	50		-	+11	-	0
4	10	20.0	-	+577→+86	-	+160→+47
	50		-	+100→-33	-	+137→+32
					After L-SULPIRIDE (100 μg kg^{-1})	
5	10	0	-57	-	(NOT TESTED)	
	50		-77	-	-	+57
	50				-	+100

* Domperidone, 10 μg kg^{-1}

† Maximum dose

→ Implies transition from a 'peak' discharge to a lower rate of discharge.

i.v.), the same level of NA infusion caused sustained excitation of the chemoreceptors.

The β_1-antagonist MET (1 mg kg^{-1}, n=1) did not alter the chemodepressant effect of infusing NA (50 µg min^{-1}; cf. Figs 5.4B and 5.5).

5.2.2.2 Infusions of isoprenaline.

ISO, infused in two experiments, caused only chemoexcitation. In one experiment infusion of 10 µg min^{-1} caused an excitation of 20% over pre-infusion control discharge (domperidone had been injected earlier in the experiment); in the other experiment there was a dose-related increase in discharge in the steady-state (Figs 5.4B and 5.6), and excitation in response to the highest rate of infusion (50 µg min^{-1}) was reduced by MET (1 mg kg^{-1}), and, again, by PROP (1.4 mg kg^{-1}, after L-sulpiride, 100 µg kg^{-1}).

5.2.2.3 Interaction of catecholamine infusion and the response to hypoxia.

Hypoxia tests were performed during infusions of NA or ISO, before and after various antagonists, in one rabbit (Figs 5.5 and 5.6). Values for mean discharge in the steady-state during air-breathing before and after commencing infusion, during 10%O_2-hypoxia (whilst infusing drugs), and peak discharge during 100% N_2-ventilation are shown in table 5.2, where data are presented in the sequence in which the experiments were carried out. Variability between tests was such that no meaningful comparisons could be made between the quantitative analyses of these tests, whether comparing discharge ($\bar{x}$ c.p.s.) directly, change in discharge expressed as a percentage

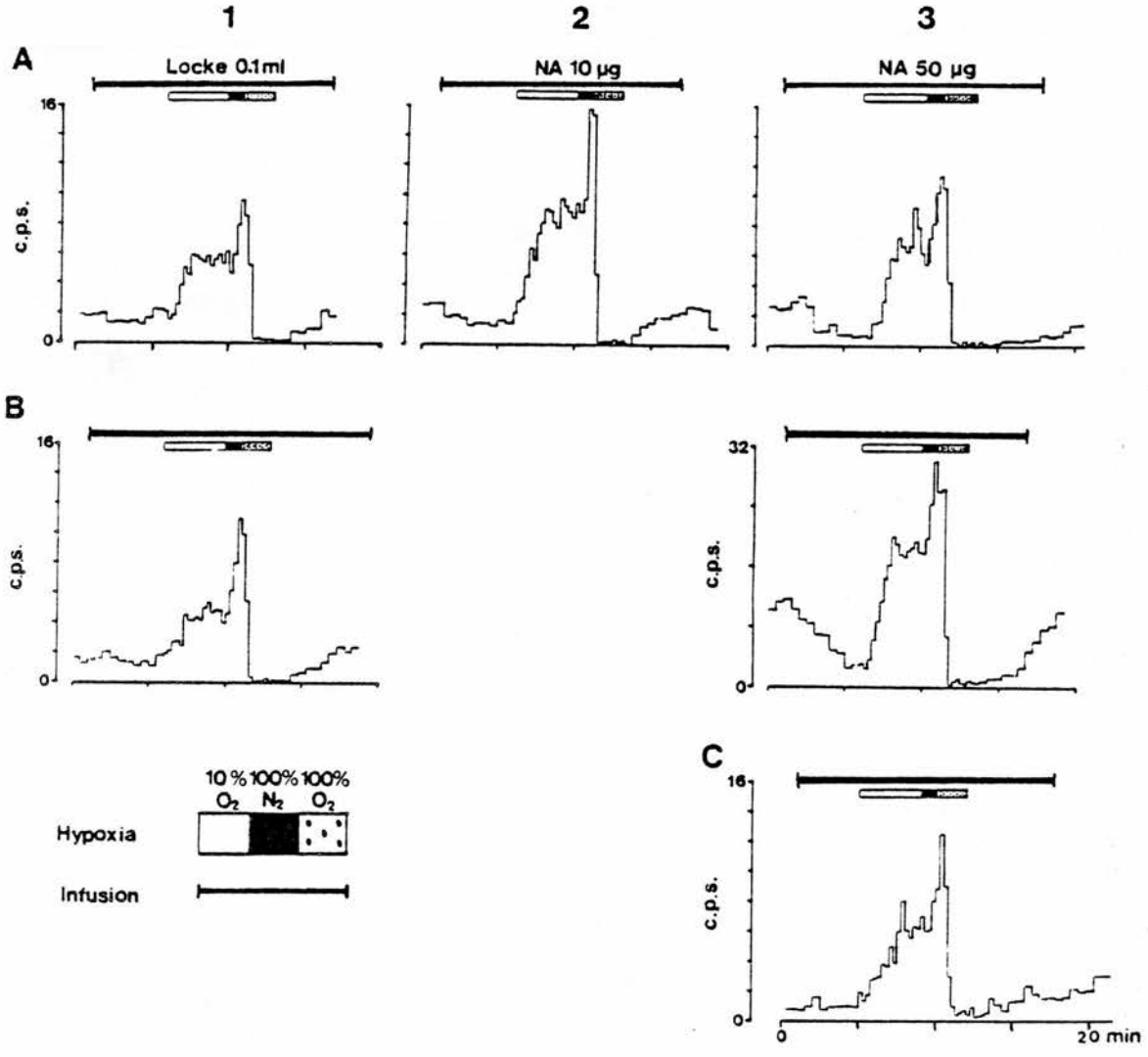


Figure 5.5. Chemoreceptor responses to ventilation with 10% O_2-90% N_2 (4 min; open bar), 100% N_2 (1 min; solid bar), and 100% O_2 (2 min; stippled bar), during infusion of Locke solution (0.1 ml min^{-1}) or NA 10 and 50 µg min^{-1}, (A) before, (B) after MET (3.5 mg kg^{-1}) and (C) after L-sulpiride (100 µg kg^{-1}). Discharge was averaged in 30 s blocks before onset of hypoxia, and in 15 s blocks thereafter.

The variability in discharge (note changes in calibration of the ordinate) did not permit meaningful quantitative comparison between tests.

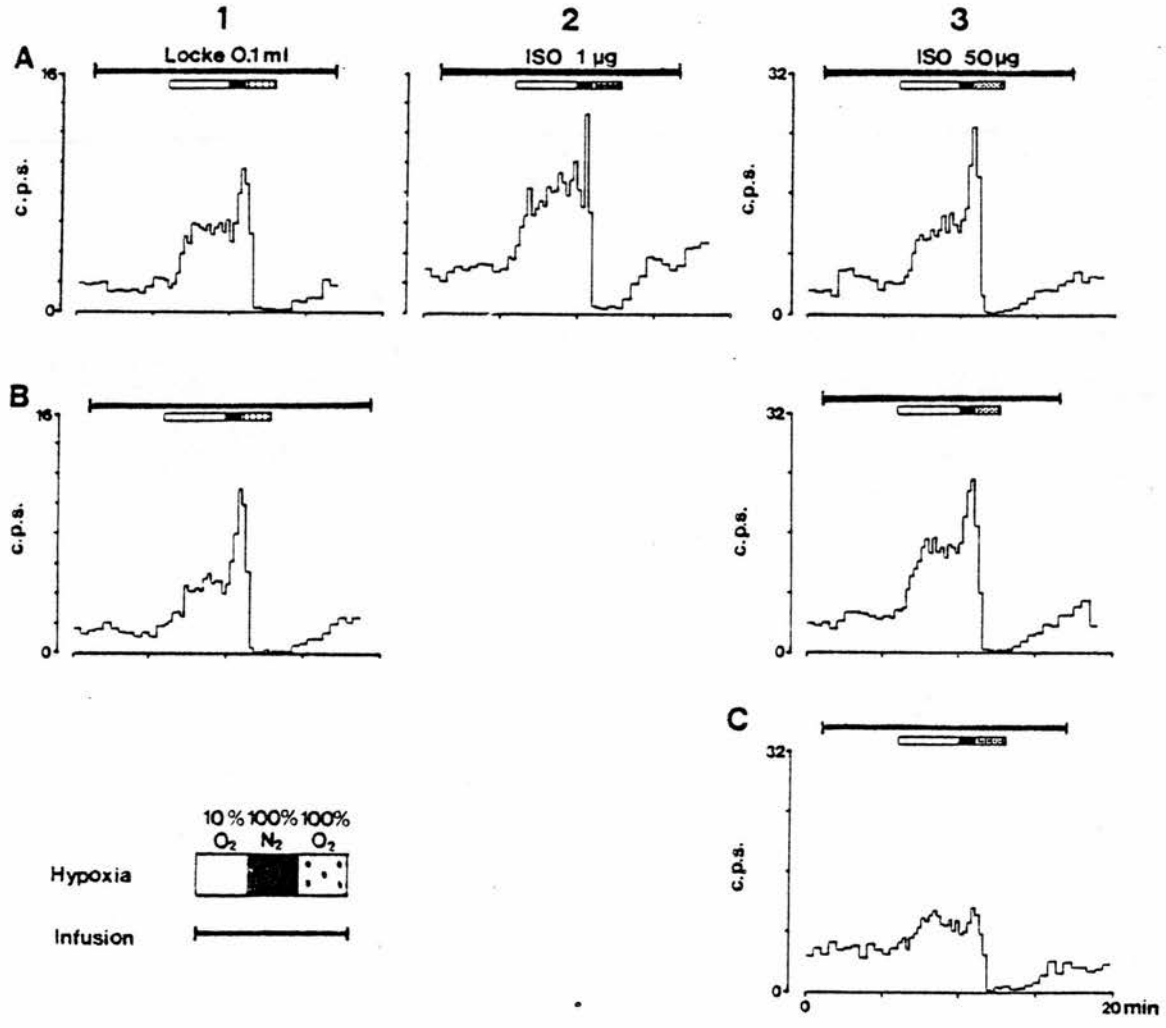


Figure 5.6. Chemoreceptor responses to ventilation with 10% O_2-90% N_2 (4 min; open bar), 100% N_2 (1 min; solid bar), and 100% O_2 (2 min; stippled bar), during infusion of Locke solution (0.1 ml min^{-1}) or ISO 1 and 50 µg min^{-1}, (A) before, (B) after MET (3.5 mg kg^{-1}) and (C) after L-sulpiride (100 µg kg^{-1}) and PROP (1.4 mg kg^{-1}). Discharge was averaged in 30 s blocks before onset of hypoxia, and in 15 s blocks thereafter.

Variability in discharge did not permit any meaningful quantitative comparison between tests.

change from each pre-infusion control, changes in discharge expressed as a percentage change from either the plateau discharge during 10% O_2-hypoxia in each test, or the plateau discharge on 10% O_2 during Locke infusion (compare with the analysis of similar experiments in the cat - Section 4). Qualitatively, ISO infusion enhanced discharge during ventilating with room air and tended to potentiate discharge during the hypoxia test. The rise in discharge elicited by infusion of ISO was reduced following MET (1 mg kg^{-1}), and also after PROP (1.4 mg kg^{-1} cf. Figs 5.4B and 5.6). NA infusion caused an initial reduction in discharge frequency, but did not alter the ability of chemoreceptors to increase their rate of firing in response to reduced arterial oxygen tension. The initial depressant effect of NA (whilst breathing air) was abolished by L-sulpiride (100 µg kg^{-1}), after which the effect of NA infusion was a slight excitation (Figs 5.4B and 5.5), as previously described.

The response of chemoreceptors to hypoxia was not obviously affected by MET or L-sulpiride (Figs 5.5 and 5.6), and after PROP only the peak discharge during ventilation with 100% nitrogen was diminished, albeit during an infusion of ISO (50 µg min^{-1}). Thus, the hypoxia response persisted after the excitatory effect of ISO was reduced, following the administration of PROP (in the presence of L-sulpiride).

5.3 Summary of results presented in section 5.

1. NA injection caused dose-related chemodepression followed by chemoexcitation. Chemodepression was blocked by the DA D_2-antagonist domperidone, after which chemoexcitation became more prominent. Chemodepression was also partially blocked by the D_2-agonist LY 141865, which may have partial agonist activity. ISO injection

caused only chemoexcitation, which was broadly dose-related.

2. Infusions of NA caused dose-related depression of chemoreceptor discharge; when large amounts of NA were infused, chemoexcitation was observed. The chemodepressant effect was abolished by L-sulpiride, a DA D_2-antagonist. In about half the experiments, only excitatory effects of infusion of NA were seen, but these infusions were carried out after prior injection of LY 141865.

3. ISO infusion caused only excitation of the chemoreceptors, and the effect was apparently reduced after MET and PROP.

4. The response to hypoxia was not obviously changed by concomitant infusion of NA or ISO, nor was the response to hypoxia markedly different after administration of β-antagonists MET or PROP, although there was evidence of blockade of the effects of ISO infusion following these drugs.

SECTION 6

DISCUSSION OF THE EFFECTS OF CATECHOLAMINES AND SELECTIVE ADRENOCEPTOR AGONISTS OR ANTAGONISTS UPON CHEMORECEPTOR ACTIVITY

SECTION 6.

DISCUSSION OF THE EFFECTS OF CATECHOLAMINES AND SELECTIVE ADRENOCEPTOR AGONISTS OR ANTAGONISTS UPON CHEMORECEPTOR ACTIVITY.

This series of experiments was performed to investigate the actions of catecholamines, particularly NA, upon carotid chemoreceptor afferent activity. It was anticipated that the use of a series of selective agonists and antagonists would allow a classification of the sub-types of receptors mediating these effects. The effects upon chemoreceptor activity of DA have been extensively reported, and it was used in these experiments as a 'marker', together with responses to cyanide or hypoxia, to confirm that a particular preparation exhibited 'typical' chemoreceptor activity, justifying the results obtained with less well-documented drugs.

Results obtained in cats and in rabbits do not suggest any marked differences, unlike the early studies of the effects of ACh in these two species (cf. Docherty & McQueen, 1979), and there appears to be no reason to treat the data obtained independently.

The response to injections of drugs is likely to resemble that evoked by transient or discrete release of neurotransmitters, an event which has not been shown definitely to occur within the carotid body. The second approach, where drugs were infused, might be taken to represent a situation where high levels of circulating catecholamines influence chemoreceptor activity. This could occur as a result of increased sympathetic and adrenal-medullary activity during exercise-stress (e.g. Galbo et al, 1975), or during altitude hypoxia in unacclimatised subjects (cf. Cunningham et al, 1965; Sharma et al,

1978).

6.1 The response of carotid chemoreceptors to NA and related drugs.

The response to injected NA was typically biphasic, chemodepression being succeeded by excitation, which could itself be divided into two distinct components. Infusions of NA caused a less complex pattern of response, which generally was a chemodepressant effect.

6.1.1 Chemodepression.

Initial chemodepression, which was dose-related, followed injection of NA at doses of 1 μg or more. This phase of the response was consistent, and has been reported earlier in a number of species (e.g. cat - Sampson, 1972; Black *et al*, 1972; Llados & Zapata, 1978b; Folgering *et al*, 1982; dog - Bisgard *et al*, 1979; rabbit - Folgering *et al*, 1982), although few attempts appear to have been made to quantify the response - which has been described as 'transient', or even 'uncommon' (Llados & Zapata, 1978b). Much of the discrepancy in the literature results, in part, from the wide range of doses used. Thus, Folgering's group reported responses to 0.5-2.0 μg NA, Llados and Zapata, ~2-7.5 μg, Bisgard and co-workers, 0.5-40 μg, and Black's group, 0.1-20 μg. From the results described in this thesis it would seem that little meaningful comparison can be made with the chemodepressor responses to NA reported by Folgering *et al* (1982) or Llados and Zapata (1978b), since the doses they used could be considered unlikely to cause a pronounced chemodepressant effect. Qualitatively, there is no disagreement with the proposition that the effect is mediated through activation of DA receptors by NA (cf. Folgering *et al*, 1982).

DA-receptors in the carotid body that mediate chemodepression

appear to be of the D_2-subtype (e.g. McQueen, 1984; Mir et al, 1984b) which are unlinked (Kebabian & Calne, 1979) or negatively linked (Creese et al, 1982) to adenylate cyclase. The D_2-receptor has been characterised from studies of DA activity in anterior pituitary mamotrophs (see Kebabian & Calne, 1979) where DA mediates inhibition of prolactin secretion independently of the stimulation of adenylate cyclase and cAMP formation. DA, apomorphine, and dopaminergic ergots are potent agonists at this receptor, and ligand binding profiles are markedly different from those of the adenylate cyclase-linked D_1-receptor typified by the bovine parathyroid gland (Kebabian & Calne, 1979).

L-sulpiride, which is selective for D_2-receptors in the pituitary (Stoof & Kebabian, 1981), abolishes DA-evoked chemodepression in the cat (McQueen, 1984) and in the rabbit (Mir et al, 1984b), where the pharmacological profile, as well as in vitro ligand binding studies, has confirmed that the receptor mediating DA-evoked chemodepression is of the D_2-subtype. In the experiments described here, L-sulpiride again abolished chemodepression evoked during infusion of NA in the rabbit (see section 5). Similarly, domperidone (DP), a selective antagonist at D_2-receptors blocks DA-evoked chemodepression in both cats and rabbits (McQueen, 1984), and in this set of experiments DP was equally effective in blocking NA-evoked chemodepression in cats and in rabbits (see Sections 3, 4, and 5). It is reasonable to conclude that NA depresses chemoreceptor activity through direct actions (or via the action of endogenously released DA) at D_2-receptors.

The possibility that chemodepression is a consequence of NA-evoked release of DA (cf. Krammer, 1978) cannot be ruled out in these experiments. Although the affinity of NA for α-receptors is some

four times its affinity for β-recepors (see Kenakin, 1981), the possible release of DA is unlikely to be the result of an α-adrenoceptor-mediated effect, since the α_2-agonist OXM caused virtually no chemodepression, and PHEN (α_1) was a weaker chemodepressant than NA, at doses equipotent in eliciting pressor responses. Similarly, although the α_2-selective antagonist RAU blocked chemodepressor responses to NA, it had a comparable effect upon depression of discharge evoked by exogenous DA. Mediation of DA release through a β_1-receptor can be ruled out simply by the fact that both NA- and DA-evoked chemodepression were markedly potentiated after the antagonists MET and BET, and the depressant effects of ISO or SAL did not differ from those of the drug vehicle.

The hydroxylated aryl-alkyl DA-derivative DOB (Tuttle & Mills, 1975) has been said not to have effects upon DA receptors (Holloway & Fredrickson, 1974), but did cause chemodepression in the experiments reported here. Minneman et al (1979a,b) showed that this drug stimulates cAMP formation, and is equipotent in inhibiting radioligand binding to β_1- and β_2- receptors. Kenakin (1981) reported DOB to be a partial agonist at α-receptors (its affinity being some twenty five times that of NA, but its efficacy only one-fortieth), with no selectivity for either β-receptor subtype ; its apparent β_1-selectivity in clinical usage stems from a net predominance of actions mediated via β_1-receptors, when opposing actions at α-and β_2-receptors mutually cancel.

Chemodepression caused by DOB could represent a non-specific action on the grounds that it is a short-lasting effect unrelated to dose. It would appear necessary to confirm whether DOB truly has no effect at DA-receptors, or whether it may be rapidly broken down with

the release of DA or DA-like metabolites. Its ability to stimulate β-receptors and adenylate cyclase does not rule out the possibility of activity at non-adenylate cyclase linked D_2-receptors, since DA itself can activate β-receptors (e.g. Goldberg, 1972), which are presumably linked to adenylate cyclase.

The full potential of the depressor response to NA is rarely achieved since, particularly with higher doses of NA, the effect appears to be cut short by the onset of marked chemoexcitation. With DA, where is no attenuation of chemodepression by an excitatory response, but a graded increase in discharge back to control frequency, with an occasional 'overshoot' that appears as a transient excitation following return of discharge to normal levels. This gradual decline of the depressant effect is only seen with NA following treatment with the antagonists MET and BET which attenuates much of the cardiovascular response to injected NA as well as the associated increase in chemoreceptor discharge.

DA is about twelve times more potent than NA in inhibiting the specific binding of [^{3}H]-apomorphine to homogenates of calf caudate tissue (Seeman et al, 1978), but given that doses of DA as low as 0.1 µg will markedly attenuate chemoreceptor discharge, whereas the threshold for the similar effect of NA is some 1-5 µg, the depressant effect of NA upon chemoreceptors is some ten to fifty times less than that of DA. Despite the differences in experimental techniques, this is perhaps not too disimilar from reports that NA is eighteen (Struyker Boudier et al, 1974) to twenty five (Woodruff, 1971) times less potent than DA in inhibiting firing of neurones from the parietal ganglion of the mollusc Helix aspersa. Caution might be advised in drawing too close a comparison, since apomorphine appears to be a DA-

antagonist in this preparation (Woodruff, 1971), contrary to its agonist activity upon mammalian chemoreceptors - cf. Docherty and McQueen (1978). At the same time, it is highly relevant that PHEN, in the molluscan preparation, does not activate DA-receptor mediated events at 2000 times the effective concentration of DA (Woodruff, 1978), and in the present study did not show any marked capacity to alter chemoreceptor activity.

Since D_2-receptor activation may actually inhibit adenylate cyclase activity (Creese et al, 1982), the reduction of chemodepression by the α_2-antagonist RAU may be a non-specific action, since the drug was applied in high doses and α-receptors involved in inhibiting adenylate cyclase activity appear to be of the α_2-subtype, whilst those that alter phophatidylinositol turnover or calcium ion levels are of the α_1-subtype (Fain & García-Sáinz, 1980). It is by no means certain that RAU does not affect DA receptors (cf. earlier reports of blockade of DA-chemodepression by α-antagonists - Section 1).

Two possible explanations of the potentiation of NA- or DA- evoked chemodepression by the β_1-selective antagonists MET and BET may be considered; first, a reduction in heart rate, and the possibility of enhanced β_2-mediated vasodilatation after β_1-blockade in vivo, could prolong the time during which injected substances remain within the carotid body and capable of reacting with other receptors unaffected by these selective antagonists. Secondly, β-antagonists are able to reduce neuronal and extraneuronal catecholamine uptake (e.g. Farrant et al, 1964; Foo et al, 1968; Iversen, 1965) independently of their blocking effects upon β-receptors. At concentrations as low as 50 μM pronethalol and PROP reduced NA uptake in the isolated rabbit heart by some twenty and forty-five percent respectively (Foo et al,

1968). The failure of an intrinsic mechanism to remove catecholamines from the environment of the chemoreceptors in the carotid body treated with β-antagonists could mean that proportionally more of the injected dose is available (also for longer) to bind with receptors mediating depressant effects.

6.1.2 Chemoexcitation.

Chemoexcitation followed the depression of discharge evoked by injections of NA, and occured also in response to injections or infusions of the agonists OXM (α_2), ISO (β_1/β_2), and SAL (β_2). When infusing NA an excitatory effect was only obtained when large amounts were infused per unit time.

Chemoexcitation appeared to be correlated with cardiovascular effects of a particular drug, with respect to latency of onset, magnitude, and duration. In the case of NA and SAL there was a separate excitatory component that appeared not to be dependent upon changes in cardiovascular activity.

6.1.2.1 Chemoexcitation associated with cardiovascular effects.

As with chemodepression, an excitatory response of chemoreceptors in situ to exogenous NA has been widely reported (e.g. Joels & White, 1968; Sampson, 1972; Llados & Zapata, 1978b; Bisgard et al, 1979; Folgering et al, 1982). Llados and Zapata (1978b) reported that the effect is blocked by the α-adrenoceptor antagonists dibenamine and phenoxybenzamine (as is the reflex hyperpnoea [Joels & White, 1968; Matsumoto et al, 1980a] caused by exogenous NA - Matsumoto et al, 1981; Yasuhara et al, 1980). Although this is consistent with the greater affinity of NA for α-receptors, in comparison with β-receptors,

the situation becomes somewhat confused in the light of reports that ADR and ISO (potent β-receptor stimulants) also cause chemoexcitation in the *in vitro* preparation of the cat carotid body (Biscoe, 1965), where, it is said, excitation is not due to vascularly-mediated vasoconstriction. Besides, the usual vascular response to ISO is vasodilatation.

Others studying the *in vitro* preparation have failed to show any immediate excitatory effect of NA or ADR upon the chemoreceptors (Eyzaguirre & Koyano, 1965). Zapata (1975, 1977) did report a slight but late potentiation of discharge in 'a few' preparations. ISO is a chemoreceptor stimulant of the cat chemoreceptors *in situ* (Llados & Zapata, 1978b; Lahiri *et al*, 1981a; Gonsalves *et al*, 1983), and again the effect has been attributed to vascular changes (e.g. Llados & Zapata, 1978b).

In addition to the *in vitro* excitatory effects of catecholamines reported by Biscoe (1965), Folgering *et al* (1982) have reported similar findings in the cat and rabbit after studying chemoreceptor responses *in situ*. These authors observed the blockade of the response to exogenous catecholamines and hypoxia by the β-antagonist PROP. Zapata *et al* (1969) attributed the effect reported by Biscoe (1965) to nerve-blockade caused by the β-antagonist DCI, rather than a consequence of β-receptor blockade. Perhaps similar explanations might be offered for the effects observed by Folgering's group (1982) since PROP has pronounced membrane stabilising activity, and has been shown to exert local anaesthetic actions upon the dog carotid body chemoreceptors (Silva-Carvalho *et al*, 1981) and carotid baroreceptors (Schultz & Zehr, 1981) *in situ*. PROP has been reported not to affect reflex responses to hypoxia in conscious man (Heistad *et al*,

1972; Patrick & Pearson, 1978) and in the dog (Kontos & Lower, 1969), nor to affect chemoreceptor responses to hypoxia or hypercapnia in the cat (Lahiri et al, 1981a).

The onset and duration of the consistent and large excitatory effect of NA, OXM, ISO and SAL reported in the present series of experiments could not be dissociated temporally from the onset and duration of the observed cardiovascular changes elicited by the drugs. Of major significance is the fact that PHEN (α_1), whilst causing hypertension as great as that evoked by NA, was virtually devoid of any effect, especially excitatory, upon the chemoreceptors. The lack of a response to PHEN suggests that there are no functional α_1-receptors in the carotid body of the cat, or that they are inaccessible to or are not activated by exogenous α_1-agonists.

It would appear, however, that α_1-mediated systemic vasoconstriction does not adequately explain the excitatory response to NA. A rise in systemic arterial pressure can contribute to an increased venous return, but also increases peripheral resistance. Although these changes contribute to an increased cardiac output (the product of heart rate and stroke volume), in the absence of changes in heart rate cardiac output will not increase. PHEN increases arterial blood pressure solely by peripheral vasoconstriction, and the drug is considered to be devoid of cardiac activity and does not increase cardiac output (e.g. Aviado, 1959). NA also increases arterial pressure by peripheral vasoconstriction (Green & Kepchar, 1959), but markedly increases cardiac output due to β-receptor mediated increases in heart rate. Chemoexcitatory responses related to an elevation of blood pressure would appear to occur only when there is a simultaneous increase in cardiac output.

The α_2-selective agonist OXM caused chemoexcitation (albeit the effect was smaller than that evoked by doses of NA equipotent in causing an increase in blood pressure), suggesting that there is some involvement of α_2-receptors in the excitatory response to catecholamines. Systemic blood pressure changes were comparable to those seen in response to PHEN or NA, but there was no obvious effect of OXM upon heart rate. It is not possible from these experiments to determine whether these α_2-receptors are a part of the sensory complex, or whether they might mediate localised vasoconstriction causing a reduced flow within some part of the carotid body.

From the limited number of measurements made (cf. Table 3.7; legends to Fig. 4.1) it appears that purely α-agonistic effects upon systemic arterial blood pressure do not result in changes in arterial gas tensions (i.e. decreased PO_2 or increased PCO_2) that will stimulate the chemoreceptors. If cardiovascular changes in response to α-adrenoceptor stimulation by exogenous catecholamines (particularly NA) are to explain the concomitant chemoexcitatory effects then it is likely that these must be mediated solely by vasoconstriction at specific localities within the carotid body causing a highly localised reduction of blood flow at some point critical for chemosensory transduction. It seems improbable that such an effect would result in chemoreceptor discharge reflecting the levels of gas tensions in the systemic circulation in any meaningful way.

Total blood flow through the carotid body may be markedly reduced by stimulating the sympathetic nerves, without altering tissue PO_2 'deep' within the carotid body, and pressumably close to the sensory complex (Acker & O'Regan, 1981). Because of this independence of capillary blood flow and local tissue PO_2 from total blood flow

through the carotid body, and considering the high threshold for OXM-evoked chemoexcitation, it seems reasonable to conclude that β-receptor activation is of some importance in evoking the large excitatory response to NA.

In the experiments described in this thesis the contribution of β_1-receptor stimulation alone determined by the use of 'selective' β_1-agonists is unresolved, given the difficulties in determining at which receptor types DOB is active, and the lack of excitatory responses to PREN, which, like DOB, cannot really be classed as a β_1-selective agonist (see later). The fact that both drugs are marketed for clinical use forms the only basis for assuming that they were biologically active. Majcherczyk (1984) also failed to detect any stimulation of chemoreceptor activity using a selective β_1-agonist, although ISO did increase discharge. Unfortunately it is not stated which agonist he used, or the experimental conditions.

The cause of vascularly-mediated chemoexcitation seen with NA would appear to be due to simultaneous α- and β- receptor stimulation; the α-receptor component causes vasoconstriction, reduced vascular capacitance and an increased venous return whereas β-receptor stimulation by NA will result in increased cardiac inotropy and, more importantly, an increase in heart rate. All these factors will contribute to a marked increase in cardiac output, which may cause an increased rate of blood flow (but possibly a decreased volume) through the carotid body. If β_2-adrenoceptors mediating vasodilatation are also stimulated the increase in rate of blood flow could be accompanied by an increased volume of flow. Also of importance during intense vasoconstriction is the increase in total peripheral resistance against which the heart must pump; this can attenuate the potential

increase in cardiac output, which may thus be a very inconstant factor between experiments. Myocardial oxygen consumption can increase after injecting NA even in the absence of an increased cardiac output because the work-load involved in maintaining blood flow in the pressence of an increased total peripheral resistance is substantially increased. The capacity of β-agonists to increase general oxygen consumption is almost certainly irrelevant, since 'oxygen consumption' reflects the metabolic activity of the whole body. Metabolic acidosis might ultimately cause stimulation of the chemoreceptors, but there was no evidence of such an effect in the acute experiments considered here.

Although the pulmonary circulation is characteristically a low resistance-high capacity circuit, intense vasoconstriction following high doses of NA is likely to occur, causing a perturbation of the normal balance of ventilation and perfusion in the lungs. Although the pulmonary arteries are less well endowed with smooth muscle than are the arteries of other beds, vasoconstriction can occur (most particularly during hypoxia - Duke et al, 1961), presumably to shift perfusion away from areas of poor ventilation. Reduced alveolar perfusion is suggested by the decrease in PaO_2 and the increase in $PaCO_2$ measured after injection or infusion of NA in these experiments. Systemic hypertension is also likely to increase small airways resistance, further reducing the capacity for gaseous exchange (Bucca et al, 1980). If the velocity of blood flow through an organ (e.g. lungs or carotid body) increases, then the equilibrium of blood gases (e.g. PO_2) is likely to be reduced (cf. Murray, 1981). These changes in blood gas tension might well be an important link between the cardiovascular changes and chemoexcitation evoked by NA.

The chemoexcitatory effects observed when blood pressure and heart rate have been increased by NA may be compared with the stimulation of chemoreceptors evoked by the β_2-agonist SAL and the mixed β_1/β_2 agonist ISO, which in these experiments both caused systemic hypotension, but an increase in heart rate. Llados and Zapata (1978b) reported that after i.v. injection of ISO in the cat chemoexcitation was correlated in amplitude and time course with the systemic hypotensive effects of the drug (a finding which is in agreement with the effects described here); these authors also observed concomitant blockade of chemoexcitation and vascular effects following PROP or DCI.

Venous return falls as vascular capacitance is increased, but the doses of $\beta_{(2)}$-agonists used in the present study also caused a simultaneous increase in heart rate, so the net effect is likely to be an increase in cardiac output. Cardiac acceleration in response to SAL may be due to activation of β_1-receptors by high doses of the drug or by partial mediation of cardiac chronotropy by β_2-receptors. A reflex response to systemic hypotension seems unlikely (parasympathetic activity is reduced in pentobarbitone-anaesthetised dogs, where i.v. injection of ADR and NA causes an increase in heart rate - Shanks 1966).

The absence of a vascularly-mediated chemoexcitatory response to catecholamine agonists after administration of selective antagonists correlates with their ability to alter part of the cardiovascular response that may contribute to an increase in cardiac output. Thus α-antagonists reduce the pressor effects that lead to an increase in venous return, β_1-antagonists reduce the evoked increase in heart rate, and β_2-antagonists reduce the hypotensive effects which may

reduce peripheral resistance.

The primary difference between NA- and SAL- mediated increases in cardiac output may be that the former increases heart rate more, and at lower doses than the β_2-agonist; ISO is a well established and potent agonist at β-receptors mediating increases in heart rate. In addition NA might cause pulmonary vasoconstriction thereby reducing the potential for gaseous exchange in the lungs. Despite the fact that $PaCO_2$ appeared to increase after both SAL and NA, PaO_2 was commonly reduced after ISO, but tended to increase after SAL. It might be concluded that changes in arterial gas tensions caused by adrenoceptor agonists contribute to the chemoexcitatory response. What remains to be clarified is the effect of flow through the carotid body, and the precise regulation of the access of blood to the chemosensory complex. The independence of glomeral tissue PO_2 from total flow (Acker & O'Regan, 1981) remains enigmatic. If β_2-receptors mediate vasodilatation within the carotid body then, especially when there is also an increase in cardiac output, the volume of blood flowing through the carotid body at a certain rate, which is also susceptible to change, may be of some importance. It is significant that Mir et al (1983) demonstrated the probable existance of β_2- (but not β_1-) adrenoceptors in the rat carotid body.

6.1.2.2 Excitatory responses to catecholamines not associated with cardiovascular changes.

This type of excitation occured in response to NA in 67% of experiments. Again, the full effect may not always be seen because it can be obscured either by the marked chemodepression evoked by NA, or by the larger secondary or delayed excitation discussed above. The

increase in magnitude of this early excitatory response after β_1-antagonists may be due to a longer period of access of the injected drug to its sites of action, presumed to result from a reduction in rate of blood flow after these antagonists. The effect could also be due to blockade of NA-uptake mechanisms by β-blocking drugs (see above). Early transient excitation was not blocked by any of the antagonists which alone or in combination initially attenuated and then, at higher doses, blocked the delayed excitatory response to NA.

It might be argued that the effect is nothing more than an injection artefact since it is not always seen, but when present, and particularly after injection of β-antagonists, it is not markedly dependent upon dose. Although the same type of excitation was sometimes seen with SAL (especially after β-blockade) and to a much lesser extent with DA (in particular after the chemodepressant effect had been blocked) or Locke injections, it was never so intense in the latter two cases as the response to NA or even SAL. In one experiment it was actually shown that the transient excitation associated with injection of Locke solution was diminished by making several injections in succession, suggesting that the effect was due to contamination of the injection cannula with residual NA. This form of excitation was not observed after injections of PHEN or OXM, and so it appears that it may be associated with β-receptor-stimulating properties. The effect was, however, not abolished by β-antagonists, whether evoked by NA or by SAL.

Bisgard et al (1979) reported an intense transient excitation in dogs following injection of DA, and preceding the chemodepressant effect of the drug. A similar effect was observed after about 45% of i.c. injections of NA in the same animals. Excitation in response to

DA was dose-dependent in this species. Although succinyldicholine and gallamine did not affect the excitatory response, D-tubocurarine (D-TC - at doses less than those required for neuromuscular block) was a potent antagonist of initial excitatory responses to DA and also 5-HT. The recovery of an excitatory response to 5-HT was more rapid than recovery of DA-evoked excitation, and it is not possible to say whether the effect is mediated by a single type of receptor sensitive to indole- and catechol- amines, or whether D-TC was acting non-selectively. D-TC did not antagonise the delayed ('vascularly-mediated') excitatory responses to NA. It is an attractive speculation that this early excitatory response to NA or DA in dogs is the same as the E_1-type excitatory effect of NA which, in the present study, was seen to occur after or towards the end of the depression of chemoreceptor discharge evoked by NA in the cat.

The E_1-excitation evoked by NA might be related to the excitatory response to DA seen after selective blockade of the depressant effects of this agonist (e.g. Llados & Zapata, 1978b; Zapata & Zuazo, 1982; Lahiri & Nishino, 1980; Nishi, 1977), or after blockade of α-receptors with dibenamine (Zapata & Zuazo, 1982). It has yet to be determined whether the excitatory effect of LSD (Nishi, 1975) is also related to this transient type of excitation, since LSD is a specific ligand for DA receptors which mediate depolarisation in some molluscan preparations (Drummond et al, 1978); the blockade by D-TC of DA-receptors mediating depolarisation in the molluscan nervous system has also been reported (Ascher, 1972).

Eyzaguirre and Monti-Bloch (1980) reported that DA-evoked chemoexcitation in the rabbit carotid body superfused in vitro was blocked by haloperidol, as was the chemodepressor response. Nishi (1977) and

Lahiri and Nishino (1980) found that the excitatory response to DA in the cat carotid body in situ emerged after treatment with haloperidol. Both Llados and Zapata (1978a) and Zapata and Zuazo (1982) found that the excitatory response to DA was not blocked by haloperidol.

It is possible that this excitatory effect of DA reported elsewhere is mediated by non-specific activity at an adrenoceptor, since the effect persisted after depressant effects of DA had been blocked, using specific antagonists. This must remain unresolved until further studies determine the nature of specific excitatory DA-receptors in the carotid body, if such exist. It is unknown whether D_1-receptors are located in the carotid body or would be activated by DA. DA D_1-receptors are held to be linked to adenylate cyclase and an excitatory response to cAMP has been reported in cats (Joels & Neil, 1968). However, α-flupenthixol, which blocks the D_1-receptor in the CNS (cf. Kebabian & Calne, 1979) does not block the excitatory response to DA in the cat (Docherty & McQueen, 1978).

DA can stimulate α-receptors directly (independently of the release of NA - e.g. Mark et al, 1970), but its potency relative to that of NA varies in different vascular beds (Waller, 1961; McNay et al, 1965; Mark et al, 1970). Stimulation of β-receptors by DA is a much weaker phenomenon; the potency of DA in the femoral vascular bed of the dog being one thousandth that of ISO, and sixty-five times less than that of NA (McNay & Goldberg, 1966).

The question of whether the chemoexcitatory effect could represent a common action of NA and DA at DA-receptors or at adrenergic receptors remains to be clarified, as does the relative importance of DβH activity which might convert DA to NA under certain circumstances.

Another finding which might be said to bear comparison with the E_1-

excitatory effect described in this thesis is the 'type I' excitation occuring in response to stimulation of the carotid body sympathetic nerves, reported by O'Regan (1981). This effect was rapid in onset, occuring 10-20 s after stimulation, and was resistant to blockade with the α-antagonists phentolamine and phenoxybenzamine; the effect was potentiated after haloperidol. Some 66% of the excitatory responses recorded were described as being of this type, which is virtually the same as the frequency of occurance of 'E_1-excitation' in response to injected NA reported here.

Conventional adrenoceptor and cholinoceptor antagonists do not appear to alter either E_1-excitation in response to exogenous NA, nor 'type I' excitation in response to sympathetic stimulation. This led O'Regan (1983) to suggest the involvement of other transmitters such as SP or VIP in the response to sympathetic stimulation. Assuming that exogenously administered drugs reach the same sites of action in the carotid body as endogenously released transmitters, it seems from the results to be presented later (Section 9) that SP itself is an unlikely candidate as the endogenous transmitter mediating sympathetic effects since it causes neither consistent nor potent excitatory effects in the carotid body. Whether it facilitates excitation in response to other transmitters in the carotid body remains uncertain (see Section 9). In addition, the response to <u>exogenous</u> NA is sufficiently intense that it appears unneccesary to invoke mediation of the effect by release of endogenous substances. It remains possible that adrenoceptors mediating the effect are atypical.

Others have also suggested the existence of 'atypical' adrenoceptors, for example, the γ-adrenoceptor proposed by Hirst & Neild (1980). In studies of the responses of the rat basilar artery to NA <u>in vitro</u>

(Hirst et al, 1982) a depolarising response to bath-application of NA was noted; cocaine potentiated the effect, and in common with depolarisations obtained by nerve stimulation, the response was not blocked by prazosin (α_1), phentolamine (α_1/α_2), or PROP (β_1/β_2). The receptors presumed to mediate the effect were activated equally well by DA, ADR, and both optical isomers of NA, but not by PHEN. Flavahan and McGrath (1980) have also shown a part of the pressor response to ADR in the pithed rat to be resistant to combined α- and β- blockade.

Bevan (1984) has criticised the proposition of a novel type of adrenoceptor by Hirst et al on the grounds that the threshold for activation by NA is generally as high as 10^{-4}M (but evoked release of NA from arteriolar nerves has been estimated to be from 0.4×10^{-3}M - Bell & Vogt, 1971 - to 10^{-2}M - Bevan et al, 1980), and the capacity of this 'receptor' to respond equally to several different catecholamines is not typical of 'conventional' receptors, which are generally held to be activated by 'selective' agonists.

What cannot be denied is that in two differing experimental approaches to the study of chemoexcitation by catecholamines, an excitatory response has been shown to resist blockade with potent conventional adrenoceptor antagonists. In one case (the results reported here) the response was evoked by exogenous NA, and in the other (e.g. O'Regan, 1981) the response was probably caused by activation of a noradrenergic pathway. The parallel with antagonist-resistant pressor responses (e.g. Flavahan & McGrath, 1980) or arteriolar depolarisation (e.g. Hirst et al, 1982) may be fortuitous, or may suggest that a more complex aspect of catecholaminergic mechanisms has yet to be fully studied and appreciated.

6.1.3 Comparison of responses evoked by injections and infusions.

Except in the case of NA (the excitatory effects of which were more marked and more consistent when the drug was injected) the response to injections of adrenoceptor agonists was much the same as the effect of infusion. Injections were normally completed within two seconds so that the amount of drug reaching the carotid body when 10 μg NA were injected is roughly equivalent to an infusion of some 300 μg min^{-1}. The doses of NA injected might be criticised as being higher than those normally used in physiological studies, and were generally greater than those employed by many other groups studying the chemoreceptors. The first few seconds of an infusion of NA might be taken to be similar to the injection of very small doses of NA; as when low doses (<1-5 μg) of NA were injected, no obvious effect upon chemoreceptor discharge could be detected. After infusions of NA which resulted in chemodepression it was noted that chemoreceptor discharge increased almost instantaneously upon stopping the infusion. The onset of chemodepression during infusions was generally not so rapid, taking a several seconds to occur, whereas with injections the depression of discharge usually commenced during the injection. The driving pressure whereby the drug is delivered to the carotid body is obviously greater during injection than during infusion, and a single, low rate of infusion (0.1 ml min^{-1}) was used so as not to bias results by applying a significant or variable pressure that could enhance flow through the carotid body.

The predominant effect of infusing NA in both cats and rabbits was a sustained depression of discharge. NA-evoked depression of the response to hypoxia or hypercapnia (which are far more intense stimuli than the more subtle changes in arterial gas tensions caused by cardio-

vascular changes evoked by infused or injected drugs) was evident after β_1-blockade had reduced cardiovascular changes in response to NA.

The profile of receptors involved in the response to infused catecholamines was similar to that deduced from the responses to injections. PHEN (α_1) was virtually inactive and OXM (α_2) was not associated with chemodepression, but with an excitatory response. The β-agonists ISO and SAL consistently caused chemoexcitation when infused, and there is no obvious explanation for the unexpected depressant effects which were occasionally observed. The excitatory effect could again have resulted from cardiovascular changes; the increase in pulse pressure usually persisted long after cessation of infusion, and background discharge was usually slightly elevated following administration of these drugs.

There is a fundamental disagreement between the present findings and the reports of Folgering et al (1982) that infusion of ADR, NA and ISO was accompanied by pronounced excitation of the chemoreceptors. These authors reported that MET (β_1) and PROP (β_1/β_2) were equally effective in shifting the dose-response curve for ISO-evoked excitation, consistent with blockade of the excitatory effect. This does not appear to allow for any β_2-effect of ISO which would be expected to remain after β_1-blockade with MET; in the present experiments SAL (β_2) was a relatively potent chemoexcitant, and Mir et al (1984a) have described β_2-binding sites in the rat carotid body, sensitive to ISO.

According to the report of Folgering et al the excitatory effects of ADR, NA and ISO were identical when infused at rates of 1 or 1.5 μg min^{-1}. In the light of the present experiments such infusions of NA would be expected depress chemoreceptor activity. These authors made

no comment of the fact that NA has predominantly α-agonist properties, or that both ADR and ISO would be expected to stimulate β_2-receptors, which NA, at these low doses possibly would not. One of their findings that is consistent with the results discussed here is that the response of chemoreceptors to NA infusion was similar to the effect of elevating $PaCO_2$. Much of the evidence drawn from the present series of experiments suggests that the enhancement of chemoreceptor discharge by catecholamines is concomitant with increases in $PaCO_2$ (and in the case of NA and ISO to a reduction of PaO_2 also). The contribution made by increased flow (during systemic hypertension) or decreased flow (hypotensive effect) is unknown.

The observation in the present experiments that reponses to catecholamine infusions could be 'reversed' when taking arterial blood samples (e.g. Fig. 4.2, where the initial depression of discharge during infusion of NA 25 or 50 μg min^{-1} is lost after the blood sample at three minutes) further suggests that there is some correlation between vascular responses and chemoexcitation evoked by catecholamines.

6.2 Interactions of catecholamines with hypoxia or hypercapnia.

The hypoxia test performed in these experiments was reproducible in cats. When discharge is plotted against time a distinctive pattern of response emerges, and these 'curves', in the absence of marked changes in background discharge, may be superimposed. If the pattern of the response is altered as a consequence of drug-administration then comparisons can be made between differing levels of discharge at each stage of the test, or of the dynamic component of the test, where chemoreceptor discharge increases in response to hypoxia.

In the rabbit the situation was less clear, since there was far less consistancy in the response to hypoxia within a single experiment - background rate of discharge tended to vary between tests performed in an experiment, and the peak discharge elicited by ventilation with 100% N_2 did not occur in all tests (but could occur in a subsequent test, which may imply that chemoreceptors are more readily exhausted than in the cat). The variation in discharge did not necessarily correlate with concomitant infusion of NA or ISO.

Ventilating cats with 10% O_2 caused a reproducible and consistent reduction in PaO_2 to ~35-40 mm Hg. Discharge during air-breathing, compared to the hypoxia steady-state level of discharge (100%), generally represented some 20-25% of that elicited during the 'maximal' response to 10% O_2, suggesting that arterial gas tensions during air-breathing and basal discharge rates were broadly comparable in different animals. During 10% O_2-hypoxia and 100% N_2-ventilation blood pressure usually decreased. At onset of ventilation with 100% O_2 blood pressure rose rapidly to ~150-200 mm Hg and heart rate increased; during this phase a transient chemoexcitation was only occasionally observed. Hyperoxia depresses chemoreceptor activity and this excitatory effect is therefore related to increased blood flow during this rapid increase in blood pressure. Whether the blood flowing through the carotid body at this time is enriched with substances or metabolites released during hypoxia remains to be established.

Changes in the pattern of response to the hypoxia test during drug infusions may be due to three factors. Firstly, direct effects of drugs upon the chemoreceptors, secondly, subtle changes in gas tensions (but the application of 10% O_2-hypoxia is likely to outweigh these), or thirdly, changes in cardiac output resulting in an increased

blood flow. Direct excitatory effects of catecholamine agonists upon chemoreceptor discharge might appear unlikely since only when relatively high amounts of ISO were infused was there evidence of an increase in the chemoreceptor response to hypoxia, even though obvious cardiovascular changes occured with the lower levels of infusion. Also, the clear depression of discharge during NA infusion at the onset of hypoxia did not appear to markedly attenuate the chemoreceptor response to hypoxia, exept after blockade of β_1-receptors.

In the present experiments it was observed that discharge increased during the infusion of ISO and when inspired CO_2 was raised. When the percentage increase in discharge evoked by CO_2 was compared to the respective air-breathing (0% CO_2) discharge, the relative effect of CO_2 was seen to be the same whether ISO was infused or not (Fig. 4.9). It is not possible to ascribe the increased level of discharge during ISO infusion solely to a direct effect of the drug upon the chemoreceptors, since not only would there have been changes in blood flow, but PaO_2 commonly fell during the infusion. The increase in chemoreceptor discharge appeared always to be concomitant with this effect.

The interaction of ISO with hypoxia remains a vexing problem. Heistad et al (1972) showed an enhancement of ventilation in response to NA or ISO infusion in man, which was abolished by PROP (in passing, it is perhaps significant that these authors saw only a small suppression, and no enhancement of ventilation during infusion of PHEN). The effect of hypoxia itself was unaltered by β-blockade, a conclusion also reached by Patrick & Pearson (1978). Wasserman et al (1979) confirmed indirectly the excitatory effect of ISO upon cat chemoreceptors; according to this report 100% O_2 attenuated hyperpnoea

in response to CN^-, but had little effect upon the ventilatory response to ISO. The authors concluded that in the absence of peripheral chemoreceptor activity central chemoreceptors respond to increased $PaCO_2$ induced by the elevated cardiac output caused by ISO, thus stimulating ventilation.

Lahiri et al (1981a) observed that injection of ISO into the femoral artery of cats caused an increase in chemoreceptor discharge within 2.5 s, peaking at 5 s (perhaps a surprisingly short latency for a direct action upon the carotid body). The chemoreceptor response to ISO was shown by these authors to be reduced by hyperoxia and augmented by hypoxia, suggesting that the response to ISO is related to intrinsic activity of the chemoreceptors at the time of injection. Evidence supplied in the report of Lahiri et al also suggests that the chemoreceptor response to ISO precedes, and is independent of cardiovascular changes. It was allowed that 'a local constrictor effect on carotid body blood vessels, which may respond to isoprenaline in a manner different from that of the rest of the circulation, cannot be ruled out'. ISO can indeed cause even systemic vasoconstriction (Butterworth, 1963) but at doses (mg) higher than those injected i.c. in the present series of experiments, and far greater than those used by Lahiri et al (1981a).

Although the addition of CO_2 to inspired air to maintain alveolar PCO_2 constant during infusion of NA in man (Barcroft et al, 1957) is required to maintain hyperpnoea in response to infusion, stimulation of ventilation by ISO is both more powerful and sustained during i.v. infusion (Cobbold et al, 1960), and is not reduced by the concomitant fall in alveolar PCO_2. It is of course impossible here to separate peripheral and central stimulatory effects upon ventilation, for even

if peripheral drive decreased with the hyperpnoeic reduction in $PaCO_2$, a central drive from stimulation of β_1-receptors - but probably not β_2-receptors - by ISO (Folgering, 1980) might compensate. Hence, evidence suggests that the ventilatory response to NA mediated by the peripheral chemoreceptors is 'self-limiting' because of the resultant fall in $PaCO_2$, but direct activation of $\beta_{(1)}$-receptors would appear also to mediate an increase in ventilation. It must be conceded that this effect, which is largely a property of the drug ISO, could be mediated at central rather than peripheral sites.

Folgering et al (1982) showed a reduced chemoreceptor response to hypoxia following β-receptor blockade. In the present experiments such an effect was seen only after relatively large doses of PROP, and even then chemoreceptor discharge never failed to increase in response to 10% O_2-hypoxia. In their experiments Folgering's group also used the β_1-antagonist MET, and found it to be as effective as PROP in attenuating the hypoxia response. Of the antagonists used in the present study, PROP, MET and ICI 118551 may be problematic because of their membrane-stabilising actions (e.g. Dohadwalla et al, 1969; Åblad et al, 1975; Bilski et al, 1979; Evangelista et al, 1981). The dose of PROP expected to exert this effect has been estimated to be 10-30 μM (Rand et al, 1976) or approximately 3-9 mg, well within the range of doses used in these experiments (PROP may also function as a 5-HT antagonist - e.g. Green et al, 1981 - which could further complicate interpretation of results obtained using it). The relatively weaker membrane-stabilising properties of MET (Åblad et al, 1975) may explain why the chemoreceptor response to hypoxia was less attenuated after this antagonist than after PROP in the present experiments.

BET has at least ten times the β_1-blocking capacity of PROP or

MET, and a longer duration of action, but it has more than twenty times less membrane-stabilising capacity as PROP (Boudot et al, 1979). This antagonist (at doses which altered cardiovascular activity and the responses to exogenous NA) caused no significant change in the response to hypoxia, and the failure of this antagonist to attenuate the effects of hypoxia can be confidently accepted as evidence that β_1-adrenoceptors are not involved in the carotid chemoreceptor response to hypoxia in the anaesthetised cat.

It is uncertain whether the attenuation of the hypoxia response after ICI 118551 resulted from damage to the carotid body caused by the acid vehicle, membrane-stabilising properties of the antagonist (which are comparable to those of PROP - Bilski et al, 1979), or to other effects.

Further experiments are necessary to determine whether an enhanced chemodepressant effect of endogenous NA after β_1-receptor blockade could account for any reduction in the response to hypoxia. Tentative evidence for such an effect is to be found in the observation that little reduction in the response to hypoxia was observed after MET when the D_2-antagonist domperidone had been previously administered. This would require that a significant ammount of NA or ADR would reach the chemosensory complex during hypoxia. Whether this would be derived from type I cells, from sympathetic terminals, or from the adrenal medulla remains to be shown.

Other reports also disagree with the findings reported by Folgering et al (1982). Mills and Smith (1983) reported a sustained increase in chemoreceptor discharge with low rates of i.v. infusion of NA (0.02 - 0.04 mg $kg^{-1}hr^{-1}$) in cats, prevented by atenolol (5 mg kg^{-1}); the response to hypoxia or asphyxia was retained after the antagonist.

Gonsalves et al (1983, 1984) showed that ISO infusion shifted the line relating chemoreceptor discharge to arterial O_2-saturation slightly to the right, and that the β-antagonists PROP, atenolol and ICI 118551 all caused a similar left-wards shift of the curve (as well as blocking the effects of ISO infusion). In no case was there any change in the slope of this line, and it was concluded that ISO did not interact with the hypoxic stimulus, but exerted actions at a site different from the O_2-sensor. The authors commented that chemoreceptor discharge declined abruptly after PROP, with a recovery to near control levels within 5 minutes, suggesting that the time interval for retesting the hypoxia response after PROP is critical. It seems unlikely that Folgering's group would have fallen into such a trap. An additional observation in the present experiments was that occasionally after PROP systemic blood pressure fell rapidly and was accompanied by a very large sustained increase in chemoreceptor discharge. This in itself suggests that the normal mechanism of chemotransduction was not compromised by the β-blocker.

6.3 Comments on the drugs used.

In an in vitro preparation of an isolated tissue it is sometimes possible to block activity selectively at specified receptors allowing the study of actions at another receptor, independently. This is not possible in the whole animal since the sensitivity of differing tissues to selective antagonists is highly variable (because of differing receptor densities, accessibilities, etc.), and marked changes in homeostasis can occur without necessarily involving the blockade of receptors whose activation interferes with the response under examination. Concentrations of drugs at different sites are

unknown and may be non-uniform in the whole animal, and reflex mechanisms or physiological antagonism can have important effects upon a particular agonist-receptor response (cf. Furchgott, 1972). Hence, the blockade of ISO-evoked hypotension may occur with lower doses of antagonists (PROP) than are required to block its cardiac actions (Shanks, 1966).

The rationale for using the 'selective' β_1-agonists DOB and PREN was to assess the effects mediated by β_1-receptors that could contribute to the complex response to NA. The effects of both drugs were markedly different, and neither can be considered to be truly selective for β_1-adrenoceptors. The actions of DOB have been discussed above; Kenakin and Beek (1980) showed the affinities of ISO and PREN for β_1- and β_2- receptors to be equal. It was determined that there were strong differences in the stimulus-response mechanisms of different tissues leading to a 'functional organ selectivity' of PREN rather than a 'receptor selectivity'. It is evident that occupation of carotid body β-receptors, (whether they be β_1 or β_2) by PREN does not lead to any marked changes in chemoreceptor activity, and β-receptor occupation by ISO, NA or SAL is either accompanied by more efficient stimulus-response coupling (adenylate cyclase activation?) at sites within the carotid body, or is more important at sites outwith the carotid body.

Results obtained with the agonist SAL may ultimately cause some problems for interpretation for, although the drug is held to be selective for β_2-receptors, it has similar binding affinities for β_1- and β_2- receptors in vitro, it is an antagonist at β_1-receptors, and it may exhibit partial agonist activity at β_2-receptors (Minneman et al, 1979a) which could cause 'desensitisation' of the preparation.

The somewhat low potency as a chemoexcitant of the α_2-selective agonist OXM may not necessarily argue against a possible involvement of α_2-receptors in the response to NA because, although the drug has five times the affinity of NA for the same binding sites, it has only 0.2-0.3 times the efficacy of NA.◆ Consequently, occupation by NA of a smaller proportion of the available binding sites is adequate for the generation of a response, in contrast with OXM.

Letts et al (1983) studied the blocking effects of PROP and MET in the cat and concluded that MET, particularly at high doses, showed no clear organ selectivity in vivo, and was 'cardioselective' (i.e. selective for β_1-receptors) at low doses only (0.1-0.5 μmol kg^{-1}, ~69-345 μg kg^{-1}). Low doses of ICI 118551 are selective for β_2-receptors, but Johns (1981) has determined that there was a significant reduction in the cardiac accelerating effect of ISO after administration of 2.9 μM of the antagonist in cats. Thus, in the present experiments the low doses of ICI 118551 (100 μg or 0.3 μM kg^{-1}) may be considered to have blocked β_2-receptors, whilst higher doses (1 mg or 3μM kg^{-1}) may have resulted in a more complete and non-selective β-receptor blockade, which, none-the-less, did not necessarily result in a loss of normal chemoreceptor responsiveness.

The antagonist yohimbine, of which COR (α_1) and RAU (α_2) are stereoisomers (cf. Weitzell et al, 1979) has been shown to have local anaesthetic properties (e.g. Lipicky et al, 1976, 1977) particularly in the heart (Azuma et al, 1978). This means that it might be necessary to apply similar reservations to data obtained after α-blockers as with β-antagonists. The selectivity of these drugs must also be treated with caution, since Langer et al (1981) demonstrated that yohimbine-derived α-antagonists differ in their potencies at α_2-

◆ Kenakin, 1984

adrenoceptor sites, but less obviously so at α_1-adrenoceptors.

A final aspect which has only recently been suggested to be of importance to adrenoceptor activity is the effect of pHa. In rats the mediation of pressor responses by α-adrenoceptors is highly susceptible to modulation by changes in acid-base balance; ADR acts predominantly through α_1-receptors at high pH, but the significance of α_2-activity increases as pHa falls (e.g. McGrath et al, 1982). These authors also demonstrated that the pressor effects of PHEN and the α_2-selective agonist xylazine decrease and increase respectively with a reduction in pHa; effects upon antagonist properties are similar.

The question to be asked in conclusion is which component of the chemoreceptor response to NA is the more important? The evidence provided by these experiments suggests that high local concentrations of NA would depress chemoreceptor activity. It is doubtful whether endogenous levels of NA would ever be sufficient to replicate the potent effects of exogenous ISO, particularly if chemoexcitation is also mediated by β_2-receptors, as suggested by the results obtained with SAL. α_2-receptors, but almost certainly not α_1-receptors, might also be involved in the excitatory response (though it is but speculative whether these are a part of the chemosensory mechanism or mediate local vasomotor activity). Even-so, in the case of NA these effects would appear to be secondary to the chemodepressant effects. Much of the excitatory response to NA can be attributed to cardiovascular changes, but general vasoconstriction with no increase in cardiac output does not appear to compromise the chemoreceptors, as evidenced by the lack of excitatory responses to PHEN (α_1).

The inability of β-antagonists to attenuate the response of

chemoreceptors to hypoxia, in disagreement with the data of Folgering et al (1982) also suggests that functional β-receptors are not important for normal chemotransduction. A phase of chemoexcitation that appears not to be dependent upon cardiovascular changes, and to be resistant to blockade by adrenoceptor antagonists raises the possibility that the carotid body contains atypical adrenoceptors which might enable the chemoreceptors to respond in a manner that cannot be correlated with the conventionally accepted properties of adrenoceptors.

SECTION 7

STUDIES ON THE EFFECTS OF 5-HYDROXYTRYPTAMINE

SECTION 7.

STUDIES ON THE EFFECTS OF 5-HYDROXYTRYPTAMINE.

7.1 Introduction.

5-HT is present in the cat carotid body (see Section 1) but its physiological role there remains to be determined. Intracarotid injection of 5-HT in anaesthetised cats produces a complex response (e.g. Black et al, 1972; Nishi, 1975; Docherty & McQueen, 1978), whilst in the in vitro preparation, no significant effect of 5-HT has been shown (Eyzaguirre & Koyano, 1965). Nishi (1975) found the response of chemoreceptors in situ to 5-HT to be unaltered by atropine or hexamethonium, thus precluding the involvement of ACh receptors, but the putative (and non-selective) 5-HT antagonists (+)-lysergic acid (LSD, which can also act as a partial agonist), methysergide, and gramine were also without effect upon the response; consequently, the receptors that might be associated with the chemoreceptor response to 5-HT remain, to date, unclassified. With the advent of more selective agonists and antagonists, a further attempt has been made to characterise the types of 5-HT receptors that might mediate the response of chemoreceptors to exogenous (and, by inference, to endogenous) 5-HT.

Fozard and Gittos (1983) and Fozard (1983, 1984a) described a new antagonist, MDL 72222 (MDL; 1αH,3αH,5αH,-tropan-3-yl-3,5-di-chloro-benzoate), which is a potent and highly selective antagonist at certain 5-HT receptors on mammalian peripheral neurones, for example, the fibres mediating the von Bezold-Jarisch reflex in the rat heart. 5-methoxytryptamine (5-MOT) is an active stimulant at a variety of 5-HT receptors (see Fozard & Mobarok Ali, 1978), but devoid of

activity at 5-HT excitatory sites on the cat inferior mesenteric (Gyermek & Bindler, 1962) and rabbit superior cervical (Wallis & Nash, 1981) ganglia, and sympathetic nerve terminals of the rabbit heart (Fozard & Mobarok Ali, 1978). It can thus be considered to be devoid of agonist activity at MDL-sensitive 5-HT receptor sites. In contrast to 5-MOT, the 2-methyl substituted form of 5-HT (2-Me 5-HT) is said to be a potent stimulant of MDL-sensitive 5-HT receptors (Humphrey, 1984).

The classification of 5-HT receptors as 5-HT_1 or 5-HT_2, on the basis of radioligand binding studies has been proposed by Peroutka and Schneider (1979). According to this classification ketanserin is a 5-HT antagonist with high selectivity for 5-HT_2 receptors, and no activity at 5-HT_1 receptors (Leysen et al, 1981). 5-HT_1 receptors have been further subdivided, and 8-Hydroxy-2-(di-n-propylamino) tetralin (8-OH DPAT) is a selective agonist at 5-HT_{1A} receptors (Middlemiss & Fozard, 1983), and 5-methoxy-3-(1,2,3,6- tetrahydro-pyridin-4-yl)1H indole (RU 24969) is a selective agonist at 5-HT_{1B} receptors (Cortés et al, 1984).

Dopamine is a potent depressant of spontaneous chemoreceptor activity in the cat (Docherty & McQueen, 1978); α-flupenthixol (0.2 mg kg^{-1}) blocks this action of DA, and higher doses (0.5-1.0 mg kg^{-1}) also block the depressant effects of 5-HT (Docherty & McQueen, 1978). It was considered that the effects upon 5-HT evoked chemodepression of domperidone, which antagonises the chemodepressor effects of DA in vivo (cf. McQueen, 1984, and Sections 3-6 of this thesis), should be examined.

7.2 Results.

Experiments were performed on twenty cats, of which seven were anaesthetised with pentobarbitone, and the remainder with α-chloralose. No obvious differences were seen in the results obtained using these two different anaesthetics, and data from the two groups were pooled.

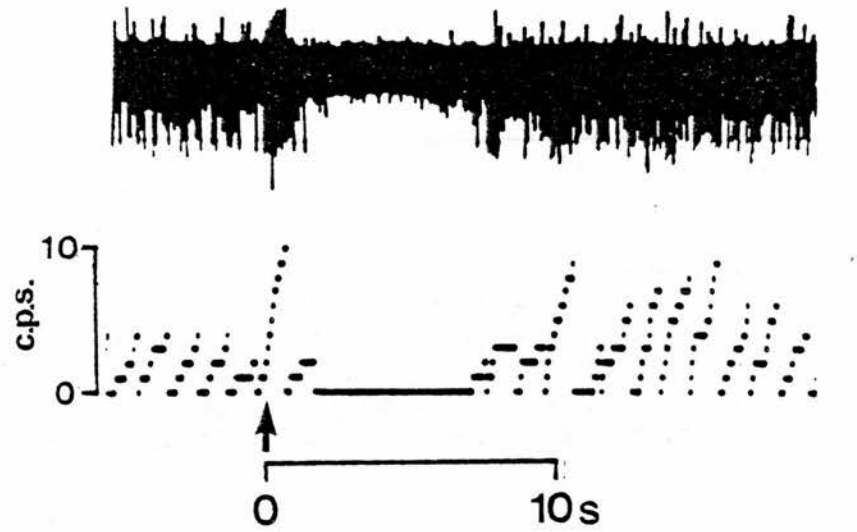
7.2.1 Effects of injected 5-HT.

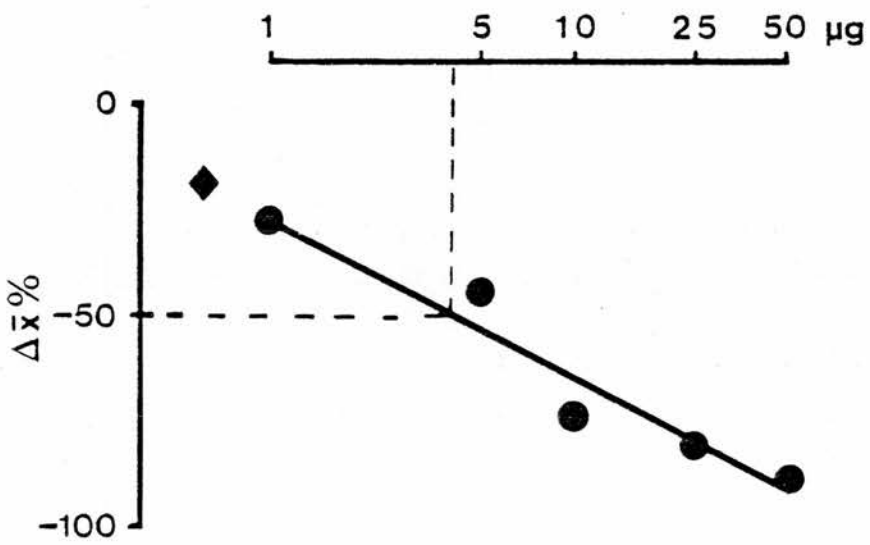
Dose-response data for the effects of 5-HT were obtained from sixteen recordings, and 5-HT (1-100 μg, injected i.c.) consistently caused a depression of spontaneous discharge, which lasted for some 3-15 s (Fig. 7.1). This chemodepression was rarely 'absolute', as occurs in response to dopamine when discharge is 'silenced' for a significant period of time. The effect was clearly dose-related in twelve (75%) of the recordings, as shown in Fig. 7.2 where the change in chemoreceptor discharge frequency in the first five seconds after injection (expressed as a percentage of the frequency during the pre-injection control period) is plotted against $\log_{10}$ dose of 5-HT. This method of expressing results was used so that a comparison could be made of data obtained from experiments with differing rates of basal discharge. In the remaining four experiments (25%), chemodepression, although present, was not obviously related to dose, and it should be noted that tachyphylaxis or desensitisation is a problem commonly encountered in studies with 5-HT; the presence or absence of such an effect in these experiments largely depends upon the interval allowed between successive injections (cf. Nishi, 1975).

The threshold for chemodepression was about 1 μg, with lesser doses producing only slight changes in discharge which were not

Figure 7.1. Neurogram showing the response of 2-3 chemoreceptors to the injection of 5-HT (100 µg i.c., at arrow). The ramped counter output below the neurogram shows the number of action potentials counted in successive 1 s intervals. Clearly shown in this figure are all three phases of the response to 5-HT - initial transient chemoexcitation, chemodepression, and subsequent (delayed) excitation.

Figure 7.2. Dose-response data showing the relationship between dose of 5-HT (log_{10} scale) and the percentage change in chemoreceptor discharge (from pre-injection control levels) that occured in the first 5 seconds after injecting the drug, and precluding the initial transient excitation. A straight line was fitted to the data by the method of least squares, and the ID_{50} determined (see broken lines). (◆) denotes the effect of injecting Locke solution (drug vehicle).


10
c.p.s.
0
0
10s


1
5
10
25
50 µg
0
Δx̄%
-50
-100

significantly different from the effects of injecting Locke solution as a vehicle control.

From dose response curves such as that shown in Fig. 7.2, the parameter 'ID_{50}' (the dose of 5-HT causing a 50% depression of spontaneous discharge in the first five seconds of response) was determined, and the mean ID_{50} (± S.E.M.) for the twelve experiments where the response was dose related was 5.8 ± 1.9 µg.

In nine of these recordings (56%), chemodepression was preceded by a transient burst of action potentials (Fig. 7.3). Both latency of onset and duration of this effect were very short, the burst usually taking place, or at least commencing, within the period of the injection. The threshold for this excitatory effect was rather greater than that of chemodepression, since transient chemoexcitation was not obvious at doses below 10 µg 5-HT. This brief phase of excitation represented an increase of up to 1000% of the pre-injection control discharge, but a clear dose-response relationship was seen in only three experiments (33% - Fig. 7.11), perhaps because of tachyphylaxis.

In those experiments (25%) where transient chemoexcitation was not observed, chemodepression was still obtained. This suggests that chemodepression does not represent a state of refractoriness after injection of 5-HT, since chemodepression also occured in the absence of any obvious initial excitatory response to 5-HT, a conclusion which is supported by the observation that a second 'burst' of action potentials sometimes occured during the 'wash' phase of i.c. injections of 5-HT, before the onset of chemodepression (Fig. 7.4).

In most experiments chemodepression was succeeded by a delayed chemoexcitation (Figs 7.1, 7.3), where the discharge frequency first returned to, and then exceeded the pre-injection control discharge.

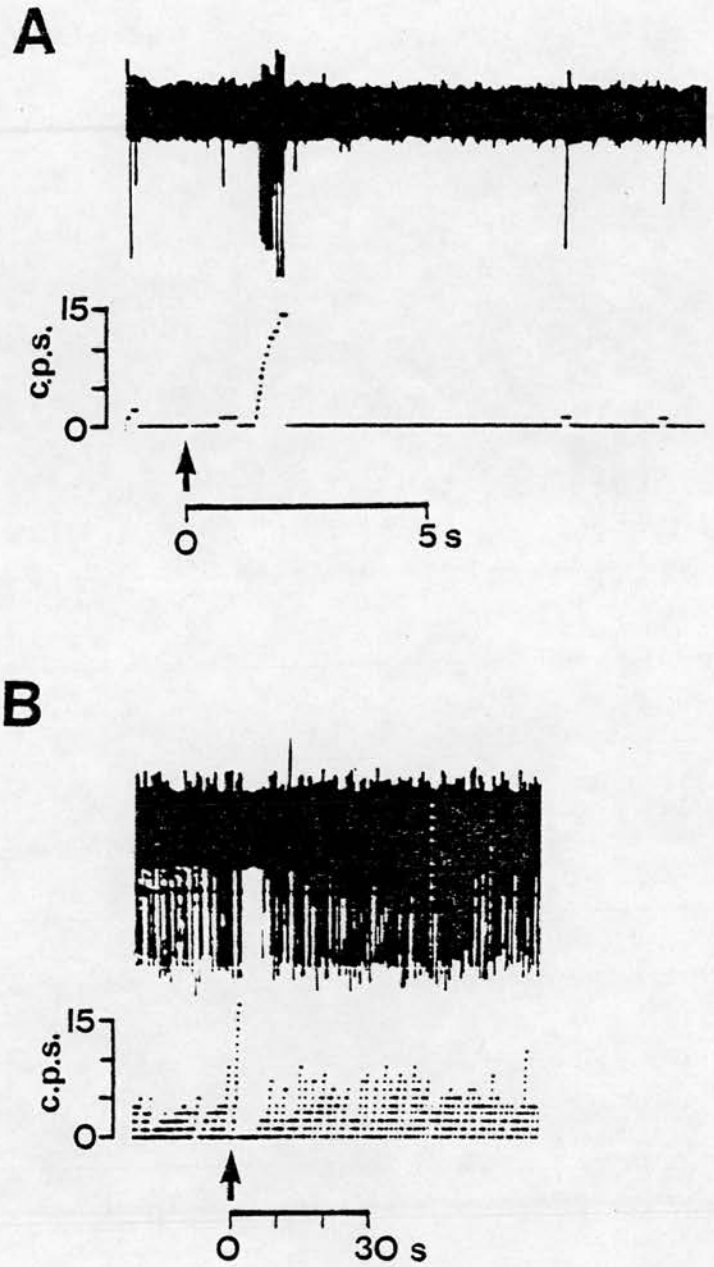


Figure 7.3. Neurograms showing the effects of the injection of 5-HT (10 µg i.c., at arrow) upon chemoreceptor discharge (3 units), and illustrating in (A) an initial burst of action potentials, followed by a period of relative depression of discharge. The same test is displayed at a slower sweep speed in (B), where a delayed phase of chemoexcitation can be seen, following chemodepression. The ramped counter output below each neurogram shows the number of action potentials counted in successive 1 s intervals.

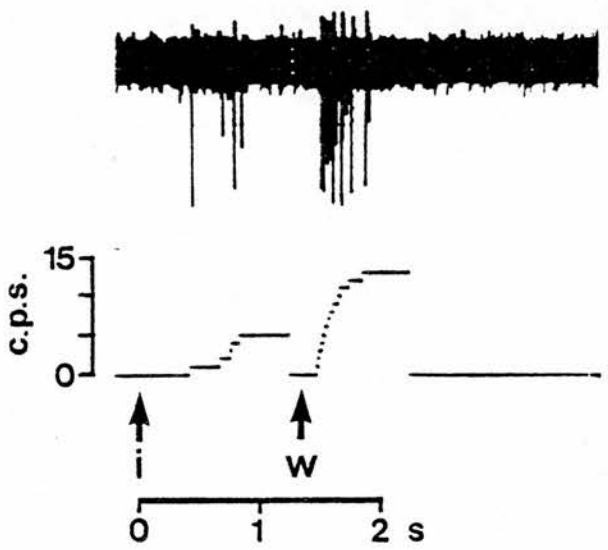


Figure 7.4. Neurogram showing the immediate response of chemoreceptors to the injection (i) of 5-HT, 25 µg i.c. A second (larger) burst of action potentials occured when the injected drug was washed in with Locke solution (w). The ramped counter output below the neurogram shows the number of action potentials counted in successive 1 s intervals.

Where present, the effect was rather prolonged, lasting some 10-60 seconds, but no consistent dose-response relationship was apparent. The onset and the duration of this effect appeared to be coincident with the systemic hypotensive response to the injection of 5-HT (see Fig. 7.14).

7.2.2 Effects of selective 5-HT agonists.

7.2.2.1 Effects of 5-methoxytryptamine.

When injected i.c., 5-MOT (1-100 µg; n=3) caused no initial transient chemoexcitation, and either no chemodepression (in one experiment), or (in two experiments) chemodepression no greater than that caused by the injection of Locke solution as a control (Figs 7.5 and 7.7). No clear secondary excitatory effect was observed, and the agonist thus appeared to have only minimal effects upon chemoreceptor discharge.

7.2.2.2 Effects of 2-methyl 5-hydroxytryptamine.

This compound was used in four experiments. In only one out of the four experiments (25%) did 2-Me 5-HT (1-50 µg, i.c.) cause an initial transient chemoexcitation; chemodepression similar to that caused by 5-HT was seen in all four experiments, although the effect was usually no greater than caused by 5-HT itself (Figs 7.5, 7.6, and 7.7). No obvious delayed chemoexcitation was observed although the agonist caused systemic hypotension comparable to that evoked by 5-HT.

7.2.2.3 Effects of the selective 5-HT$_1$ agonists, 8-OH DPAT & RU24969.

Both these agonists were applied in the same two experiments, and

Figure 7.5. Neurograms showing responses to i.c. injection (at arrows) of 5-HT (10 μg), 2Me 5-HT (25 μg), 5-MOT (10 μg), 8-OH DPAT (10 μg) and RU 24969 (25 μg). Responses to the same doses of 5-HT and 2Me 5-HT after MDL, 1 mg kg^{-1} are also shown. The typical triphasic response to 5-HT is clearly demonstrated, and the usual changes are seen after the antagonist; the response to 2Me 5-HT is similar, but the initial transient excitation appears to be absent. 5-MOT was devoid of significant influence upon chemoreceptor activity, and 8-OH DPAT and RU 24969 were potent depressors and excitors respectively of chemoreceptor activity. A ramped counter output beneath each neurogram shows the number of action potentials counted in successive 1 s intervals.

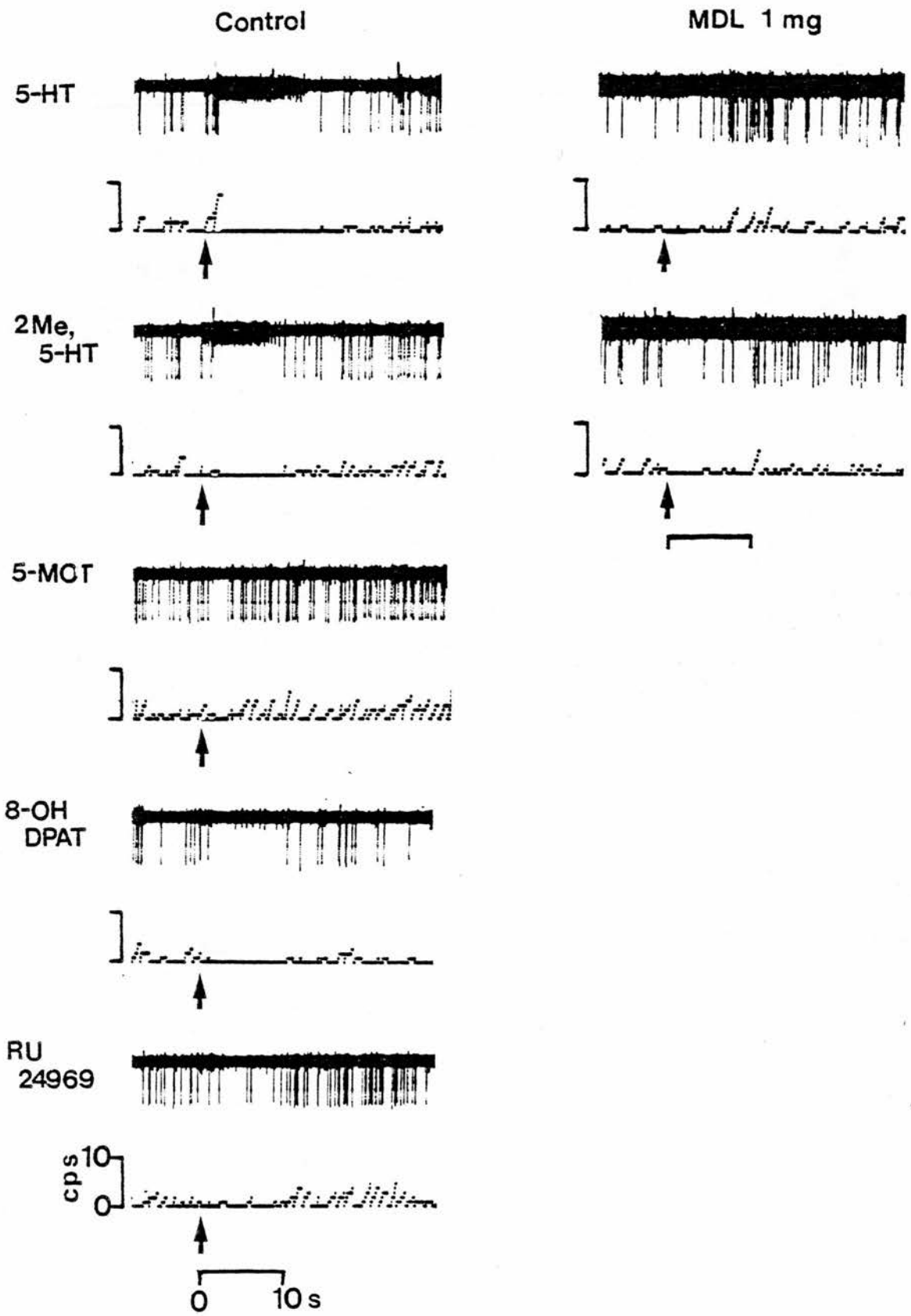

Control
MDL 1 mg
5-HT
2Me,
5-HT
5-MCT
8-OH
DPAT
RU
24969
cps 10
0
0
10 s

Figure 7.6. Neurograms showing responses to i.c. injection (at arrows) of 5-HT (10 µg), 2Me 5-HT (10 µg), RU 24969 (25 µg) and 8-OH DPAT (25 µg). Responses to the same doses of agonists, with the exception of 2Me 5-HT, are shown after MDL 100 µg kg^{-1}. The typical triphasic response to 5-HT is clearly demonstrated, and the usual changes are seen after the antagonist; the response to 2Me 5-HT is similar, but the initial transient excitation appears to be much smaller. 8-OH DPAT and RU 24969 were potent depressors and excitors respectively of chemoreceptor activity, and their affects were not markedly altered after the 'neuronal' antagonist. A ramped counter output beneath each neurogram shows the number of action potentials counted in successive 1 s intervals.

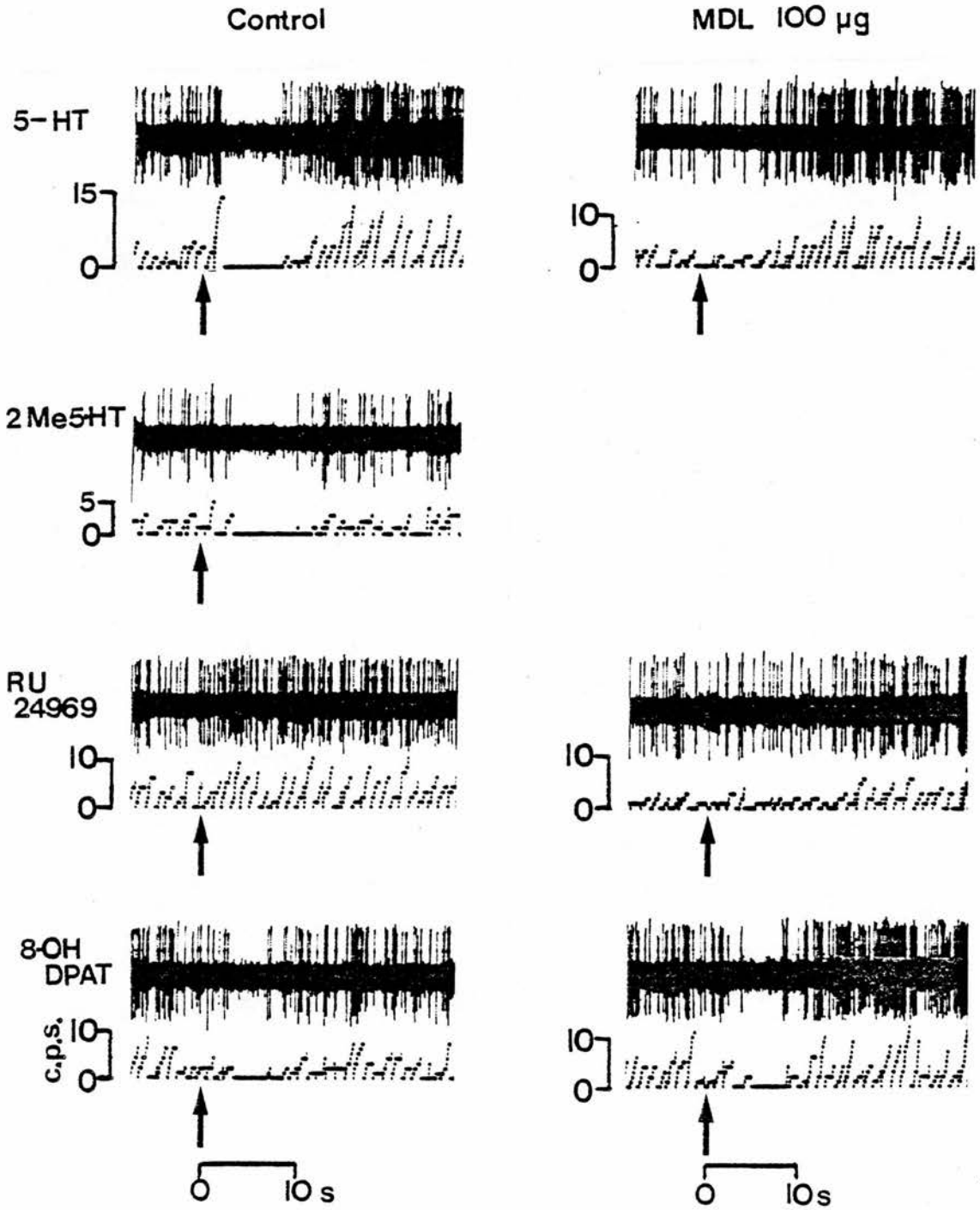
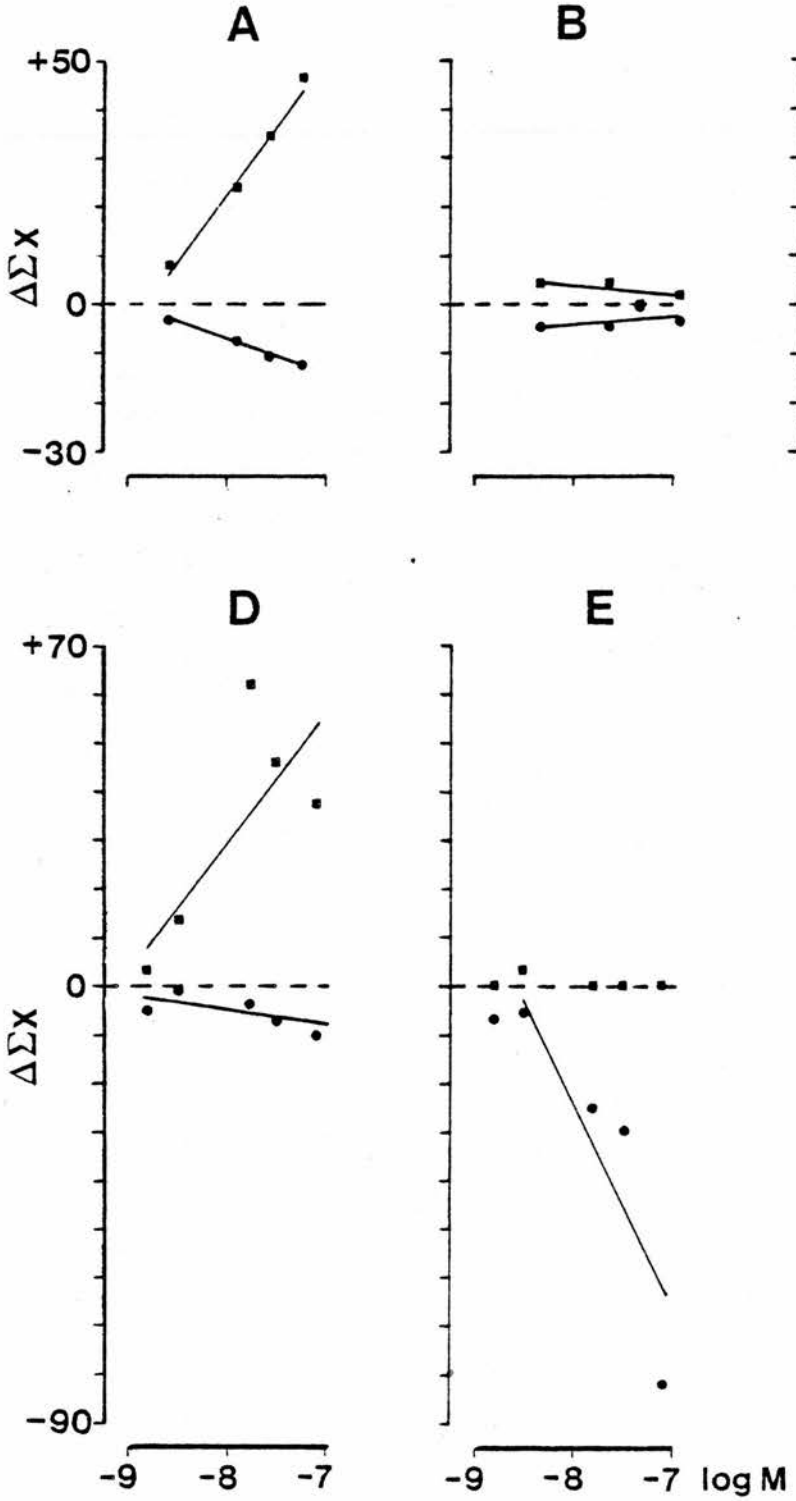

Control
MDL 100 µg
5-HT
15
0
10
0
2 Me5HT
5
0
RU 24969
10
0
10
0
8-OH DPAT
c.p.s.
10
0
10
0
0
10 s
0
10s

Figure 7.7. Dose-reponse data from a single experiment showing the chemodepressant effects (●) and excitatory effects (■) of injecting (A) 5-HT, (B) 5-MOT, (C) 2Me 5-HT, (D) RU 24969, and (E) 8-OH DPAT. Changes in chemoreceptor discharge were integrated with respect to pre-injection control discharge, ($\Delta\Sigma x$), and plotted against dose, using the same log-molar scale. Lines were fitted to the data using the method of least squares.


A
B
C
+50
0
-30
ΔΣX
D
E
+70
0
-90
ΔΣX
-9
-8
-7
-9
-8
-7
log M

their effects were markedly different.

RU24969 had no effect upon systemic blood pressure, but caused a marked, dose-related chemoexcitation (Figs 7.5, 7.6, and 7.7), following a transient depression of chemoreceptor discharge not markedly different from that caused by injection of the drug vehicle. The latency of onset of the excitatory effect was only a few seconds, but the duration of the effect was much longer than that of other excitatory responses evoked by any of the 5-HT agonists studied.

8-OH DPAT caused neither initial transient nor delayed chemoexcitation, but did evoke chemodepression similar to or greater than that observed with 5-HT (Figs 7.5, 7.6, and 7.7). This agonist caused a prolonged and progressively larger 'step-wise' decrease in blood pressure as the dose was increased.

7.2.3 Experiments with novel 5-HT antagonists.

7.2.3.1 Effects of MDL 72222.

Intracarotid injection of MDL (10-100 μg kg^{-1}) caused a depression of chemoreceptor discharge which was followed by a delayed increase in frequency (Fig. 7.8); transient chemoexcitation during the injection MDL was never observed, and systemic blood pressure was not noticeably affected by the antagonist.

In seven recordings MDL (10 μg kg^{-1} i.c.) caused the dose response curve for 5-HT-induced chemodepression (first five seconds after injection) to shift upwards, and to the right (Fig. 7.9), and the mean ID_{50} was increased to 49.4 ± 33.6 μg ($n=7$; $P < 0.05$ with respect to controls [see above], Wilcoxon two sample test, two-tailed).

When a dose of 100 μg kg^{-1} was given (eight recordings, in six of

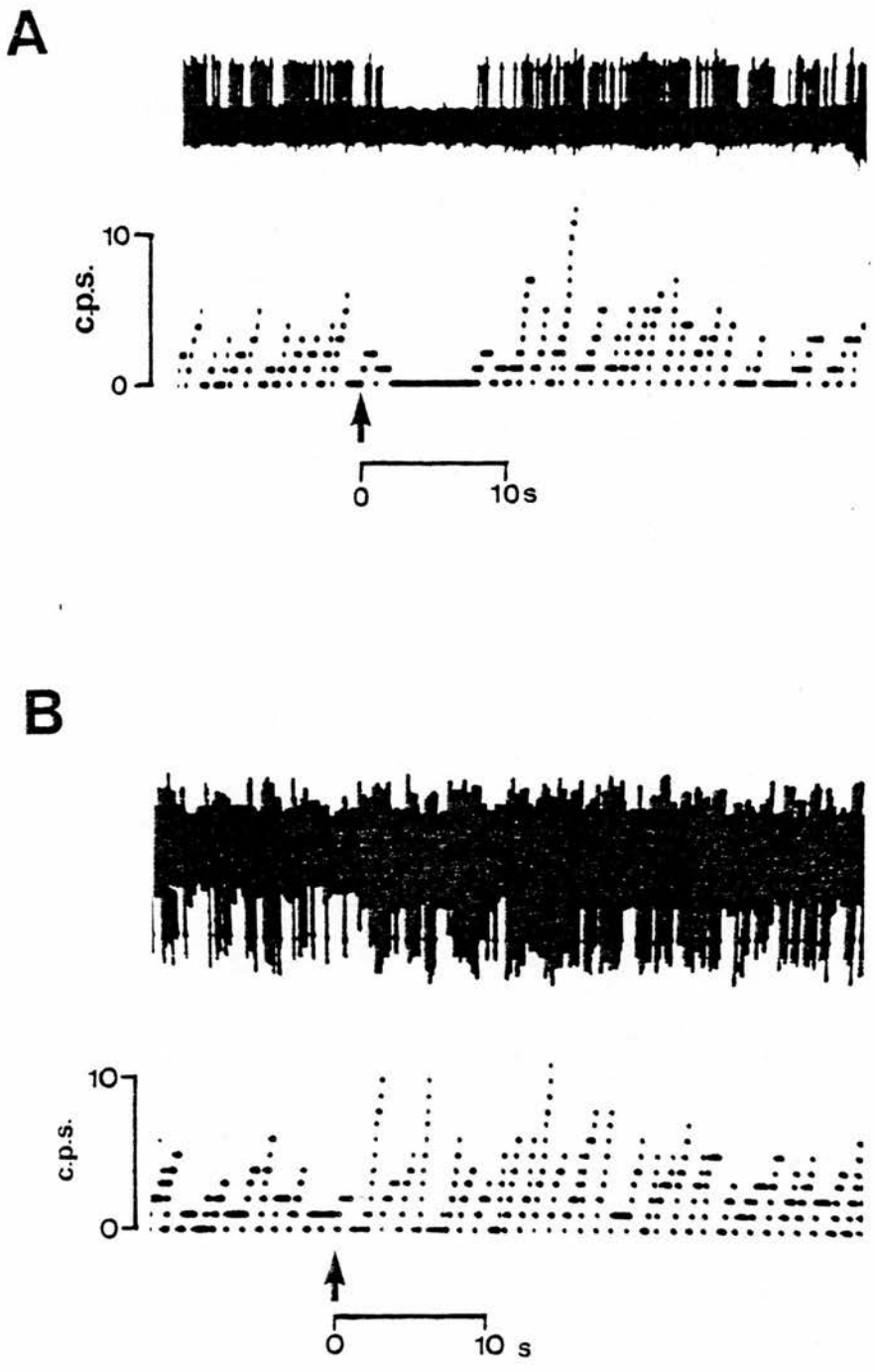


Figure 7.8. Neurograms of chemoreceptor discharge illustrating the response to the injection, (at arrow) of (A) MDL 72222 (100 μg kg^{-1}, i.c.), and, from a separate experiment, (B) ketanserin (100 μg kg^{-1}, i.c.). The ramped counter output below each neurogram shows the number of action potentials counted in successive 1 s intervals.

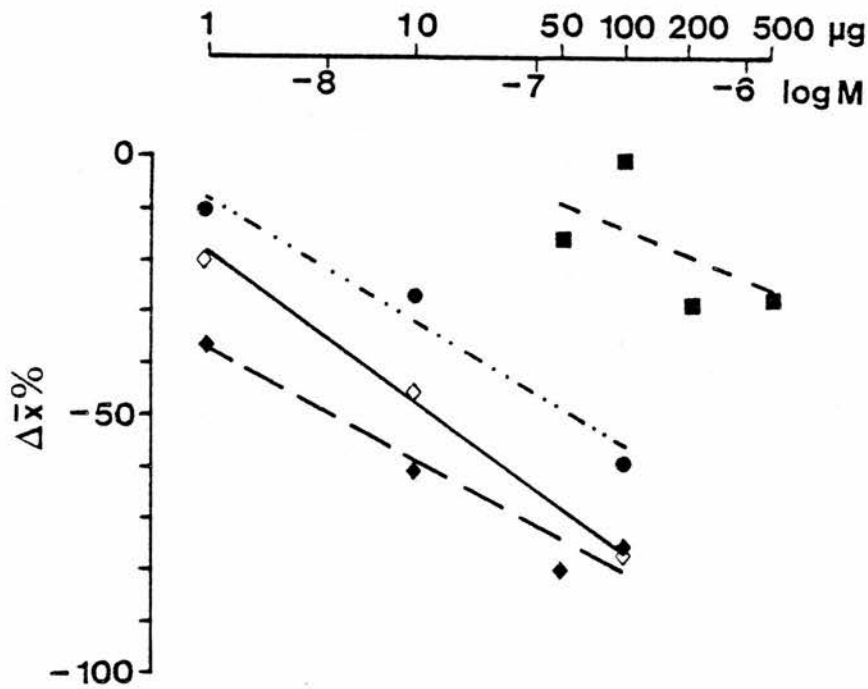


Figure 7.9. Chemodepressant effect of 5-HT (expressed as a percentage change from pre-injection control discharge) injected before (◇——◇) and after MDL 72222, 1 (◆ - - ◆), 10 (●···●), and 100 (■ - - ■) µg kg^{-1}. Although the low dose of antagonist appeared to potentiate the chemodepression evoked by 5-HT, higher doses caused progressive rightward shifts in the dose-response curve, increasing the ID_{50}.

which it followed the lower dose) there was a further upwards, and rightwards shift of the dose-response curve, and the ID_{50} was increased to 638 ± 408 µg in five of the recordings (63% - Figs 7.9, 7.10). This increase was, again, significant at the 0.05 level. In the remaining three recordings (37%) a dose-dependent chemoexcitation was obtained in the first five seconds after the injection of 5-HT (e.g. Fig. 7.10), and no chemodepression occured unless very high doses of 5-HT (250-1000 µg) were injected. This chemoexcitation never attained a 50% increase above control and it appeared more valid to calculate an ED_{30} (dose of 5-HT causing a 30% increase in discharge frequency above control), which was 103.1 ± 9.0 µg (n=3).

In the nine experiments where transient chemoexcitation was evoked by 5-HT, this part of the response was substantially reduced (totally abolished in two recordings - 22%) by MDL, 10 µg kg^{-1} (Fig. 7.11). Increasing the dose of antagonist to 100µg kg^{-1} further reduced or abolished this part of the response (Figs 7.11 and 7.12).

The delayed or secondary chemoexcitation generally increased in magnitude, and became more rapid in its onset after MDL (10-100 µg kg^{-1}; Fig. 7.13). This phase of increased chemoreceptor discharge thus no longer appeared to be correlated with systemic hypotension. The magnitude and latency of onset of the hypotensive response to 5-HT was not obviously altered by the antagonist.

Chemodepression evoked by 2Me 5-HT was slightly reduced after MDL in the one experiment where studied, although the depressant effect of 5-HT itself was more obviously attenuated (Fig. 7.5)

The effects of 8-OH DPAT after MDL (100 µg kg^{-1}) were studied in only one experiment, where it appeared (Fig. 7.6) that chemodepression evoked by 8-OH DPAT resisted blockade by MDL, even though the

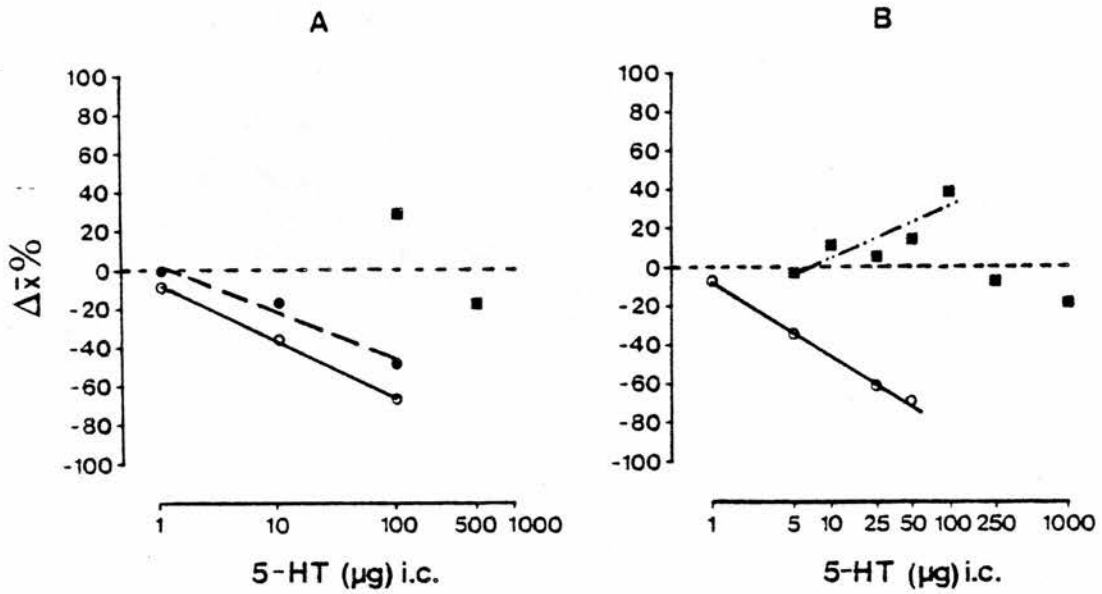


Figure 7.10. (A) Chemodepressant effect of 5-HT injected before (O——O) and after (● - - ●) MDL 72222, 10 µg kg^{-1}, i.c. An additional dose of antagonist (100 µg kg^{-1}, i.c.) caused a further shift of the dose-response curve upwards and to the right (■). In a separate experiment (B) the higher dose of antagonist (100 µg kg^{-1}) completely abolished 5-HT-evoked chemodepression (■-·-■), and slight chemoexcitation was obtained in response to the lower doses of 5-HT Lines were fitted to the data using the method of least squares.

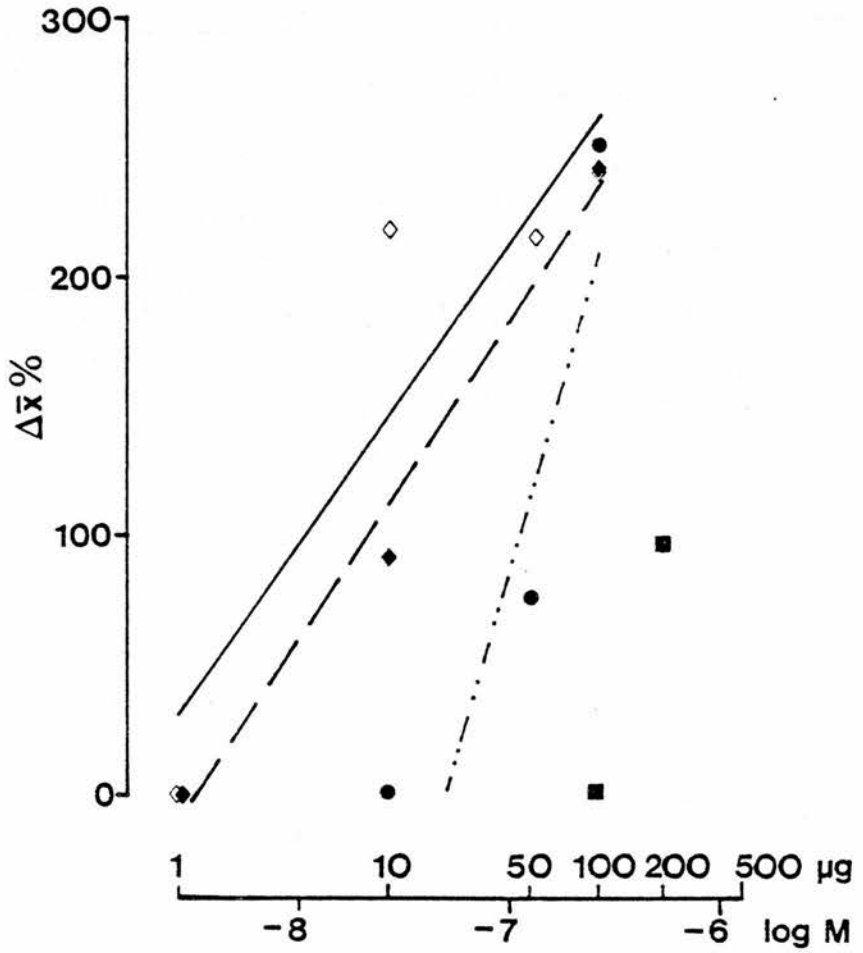


Figure 7.11. Discharge during the initial transient chemoexcitation ('burst') expressed as a percentage change from pre-injection control discharge, and plotted as a function of dose of 5-HT, before (◇——◇) and after MDL 72222, 1 (◆ - - ◆), 10 (●-·-·-●) and 100 (■) µg kg^{-1}, i.c. Lines were fitted to the data using the method of least squares.

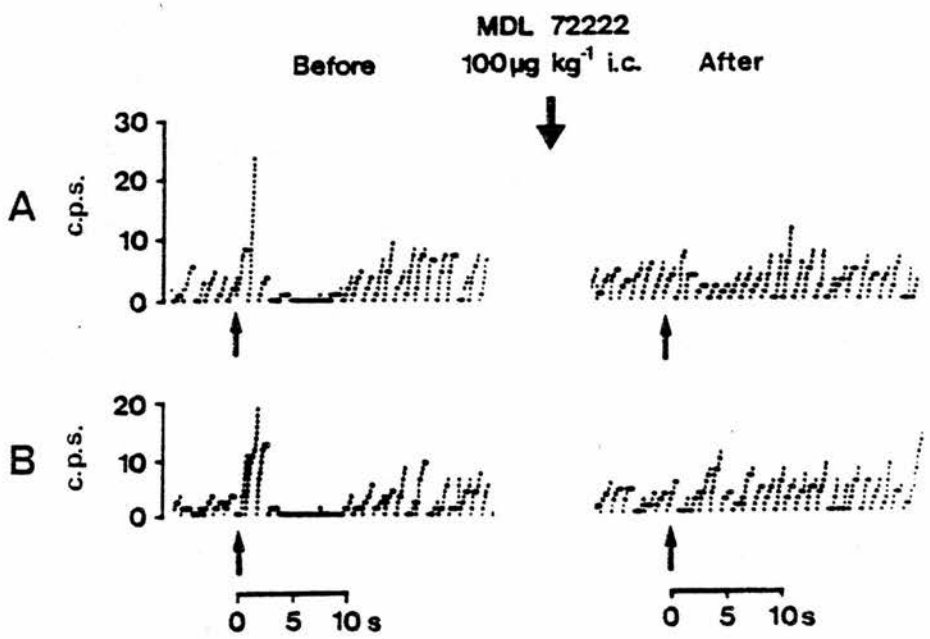


Figure 7.12. Responses of chemoreceptors (multiple-unit recording) to i.c. injections at arrows of 5-HT 5 µg (A), and 25 µg (B), before and after injecting MDL 72222 100 µg kg^{-1}, i.c. The initial transient chemoexcitation and the subsequent depression of discharge were both virtually abolished by the antagonist, after which chemoexcitation became more evident in the first five seconds of the response to 5-HT injection. Each ramped counter output shows the number of action potentials counted in successive 1 s intervals.

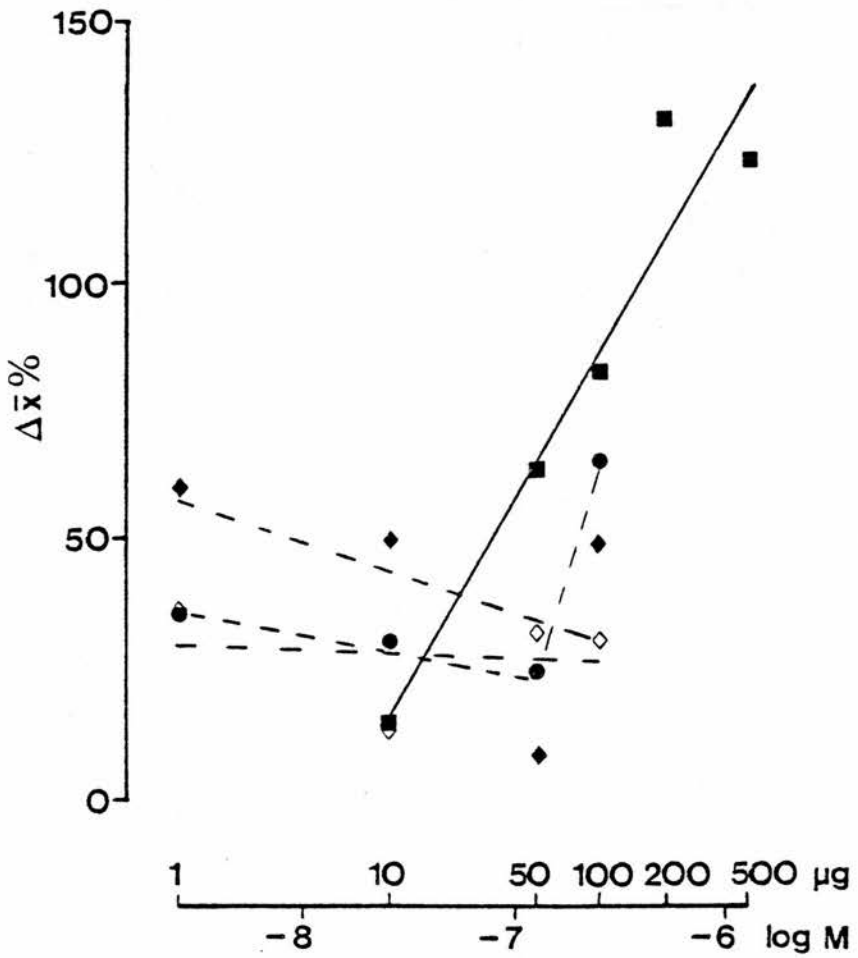


Figure 7.13. Delayed chemoexcitation expressed as a percentage change from pre-injection control discharge, and plotted against dose of 5-HT before (◇- - -◇) and after MDL 72222, 1 (◆- - -◆), 10 (●- - -●), and 100 (■——■) µg kg^{-1}, i.c. Delayed excitation became prominent, and more clearly dose-related, after the high dose of antagonist. Lines were fitted to the data, as appropriate, using the method of least squares.

chemodepressant effect of 5-HT was attenuated.

7.2.3.2 Effects of the antagonist ketanserin.

The effects of ketanserin alone were studied in five experiments. Intracarotid injection of ketanserin (100μg kg^{-1}) caused an increase in chemoreceptor discharge lasting some 10-30 s (Fig. 7.8), accompanied by a much more prolonged fall in systemic arterial blood pressure, which may indicate a change in tonic vascular tone. The injection of this antagonist caused neither chemodepression nor any bursting pattern of chemoexcitation.

5-HT was the only agonist studied after injection of ketanserin. In three of these recordings where there was an initial transient excitation in response to 5-HT, the effect was unaltered by ketanserin (e.g. Fig. 7.14). The marked chemodepression appeared also to be unchanged after administration of the 5-HT_2 antagonist, but there was a small increase in the $\overset{\text{mean}}{ID_{50}}$ for the 5-HT-induced chemodepression, to 14.2 ± 5.0 μg (n=4; $P < 0.05$). This represents a small but statistically significant decrease in the average response after ketanserin, although substantially less marked than the antagonism of chemodepression caused by even 10 μg kg^{-1} MDL, and in some tests ketanserin showed little or no antagonism of 5-HT-evoked chemodepression, as can be seen from inspection of Fig. 7.14.

In all five recordings where tested both the delayed increase in chemoreceptor discharge and the hypotensive effect of 5-HT were substantially reduced, or abolished by ketanserin (Fig. 7.14).

In five experiments where ketanserin (100 μg kg^{-1}) was injected after MDL (100 μg kg^{-1}), any chemoexcitatory response to injected 5-HT was blocked, and the plot of the response occuring in the first five

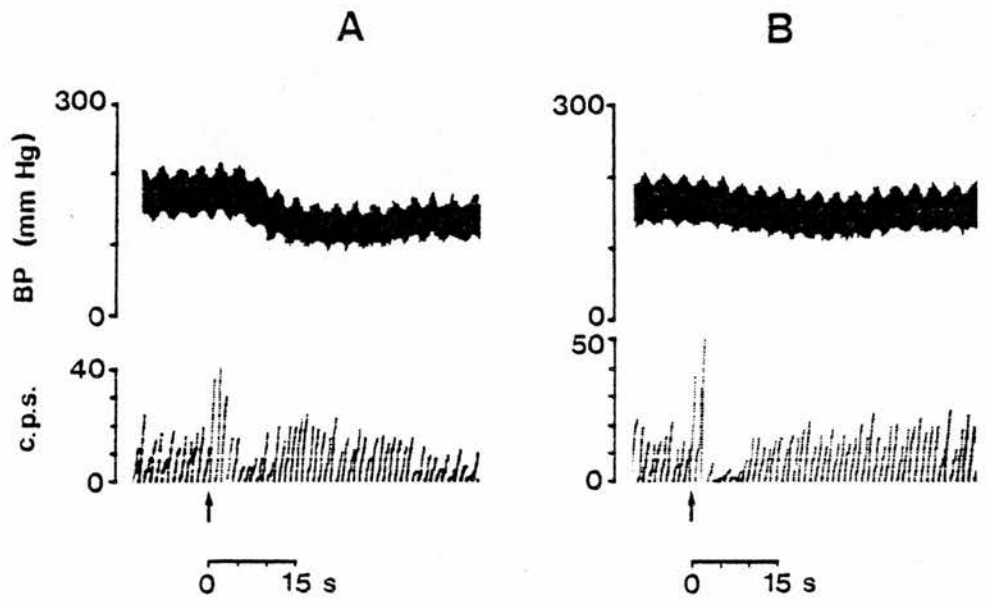


Figure 7.14. (A) Injection of 5-HT (10 µg i.c. at arrow) caused typical changes in chemosensory discharge (lower trace - count of action potentials in successive 1 second intervals), and also caused systemic hypotension (upper trace).

(B) After ketanserin (100 µg kg^{-1}, i.c.) the same dose of 5-HT had much less effect upon blood pressure, and there was less delayed chemoexcitation (~10-15 s after injecting 5-HT). Both initial transient excitation and chemodepression were relatively unaffected by this antagonist.

seconds after injection against $\log_{10}$ dose produced a line of such shallow slope that values for ID_{50} or ED_{30} could be obtained only by extrapolation far beyond the range of doses that could feasibly be used in these experiments, and were therefore not considered to be meaningful. Similarly, when MDL (100 μg kg^{-1}) was administered after ketanserin, in five experiments, there was no longer any obvious chemoreceptor response to injected 5-HT.

7.2.4 Effects of the dopamine D_2-antagonist domperidone.

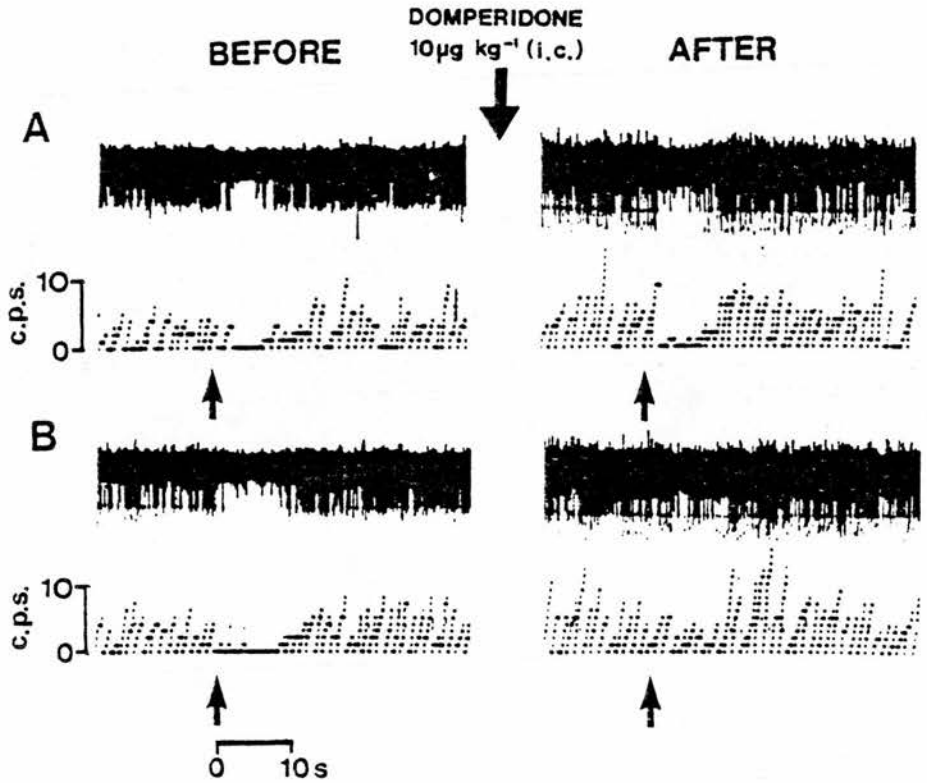
This antagonist had no effect upon 5-HT-evoked chemodepression, or any other part of the response to 5-HT (Fig. 7.15). The doses applied (10-100 μg kg^{-1}, i.c.) were adequate to block the chemodepression evoked by injection of DA (0.1-10 μg i.c.). No effect of domperidone upon the 5-HT response was observed whether the injection was made before (n=4) or after (n=4) the application of the 5-HT antagonists reported above. ID_{50} values for 5-HT-induced chemodepression obtained after domperidone in the absence of other antagonists were 10.8 ± 2.5 μg (n=4) and 8.1 ± 3.1 μg (n=2) for the 10 and 100 μg kg^{-1} doses respectively ($P > 0.05$ when compared with controls). Conversely, the chemodepressant effect of DA (0.1-10 μg i.c.), which was obtained in all recordings, was unaffected by either ketanserin or MDL (Fig. 7.16).

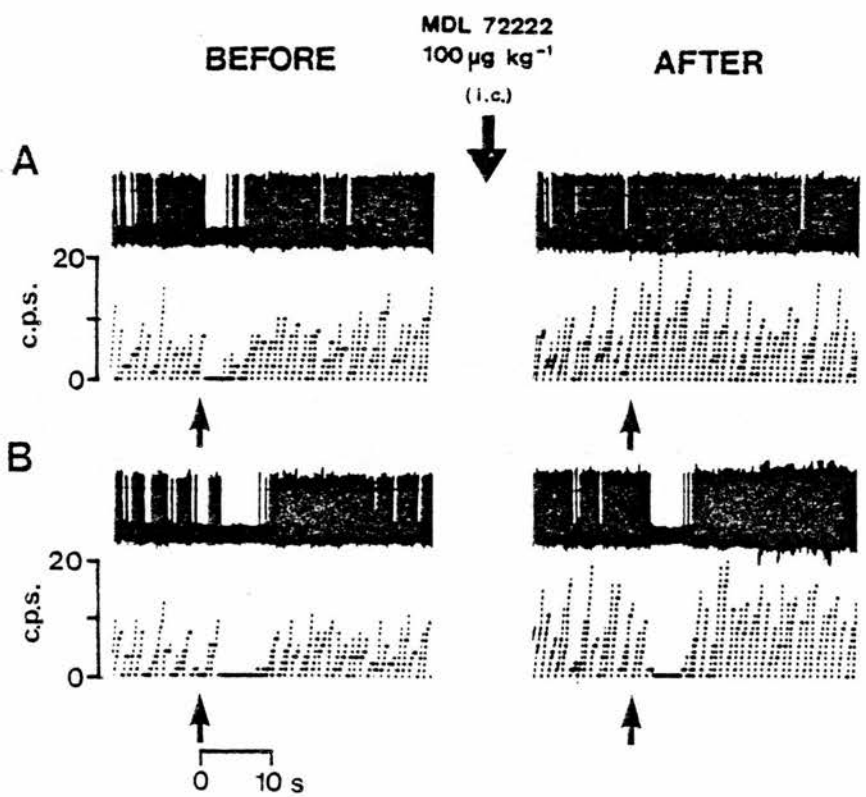
7.2.5 Effects of antagonists upon the responses to physiological (hypoxic) stimulation.

The effect of hypoxic stimulation (4 minutes ventilation with 10% O_2:90% N_2) was studied in fourteen experiments. Figure 7.17 shows the responses to hypoxia obtained before and after the injection of

Figure 7.15. Neurograms showing the responses of chemoreceptors to injections (at arrows) of (A) 5-HT (10 μg i.c.) and (B) DA (1 μg i.c.) before and after administering the antagonist domperidone (10 μg kg^{-1} i.c.). The antagonist greatly reduced DA-evoked chemodepression, without appreciably altering the response of chemoreceptors to 5-HT. A ramped counter output below the neurograms shows the number of action potentials counted in successive 1 s intervals.

Figure 7.16. Neurograms showing the responses of chemoreceptors to injections (at arrows) of (A) 5-HT (25 μg i.c.) and (B) DA (1 μg i.c.) before and after administering the antagonist MDL 72222 (100 μg kg^{-1} i.c.). The antagonist virtually abolished 5-HT-evoked chemodepression, whilst that caused by injection of DA was hardly altered. A ramped counter output below each neurogram shows the number of action potentials counted in successive 1 s intervals.


DOMPERIDONE
10µg kg⁻¹ (i.c.)
BEFORE
AFTER
A
B
c.p.s.
10
0
0
10 s


MDL 72222
100 µg kg⁻¹
(i.c.)
BEFORE
AFTER
A
B
c.p.s.
20
0
0
10 s

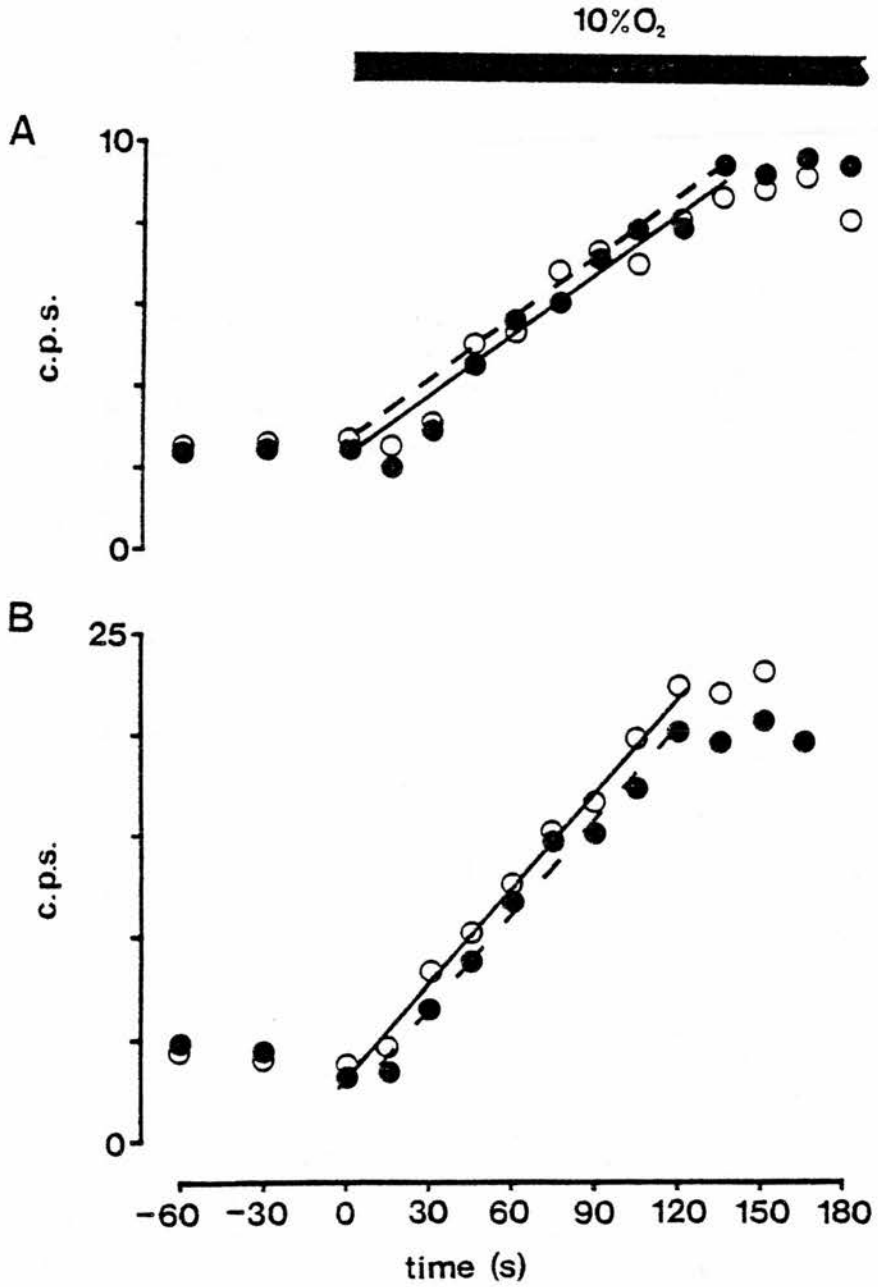


Figure 7.17. The increase in chemoreceptor discharge caused by ventilating with 10% O_2:90% N_2 for 4 minutes (solid bar), commencing at 0 s. Discharge was averaged in consecutive 15 s intervals before (O———O) and after (● - - ●) injecting (A) MDL 72222 (100 μg kg^{-1}, i.c.) or (B), in a separate experiment, ketanserin (100 μg kg^{-1}, i.c.). Lines were fitted to the data in the ranges shown using the method of least squares. Neither antagonist had any significant effect upon the response of chemoreceptors to hypoxia.

MDL (100 μg kg^{-1}; A), or ketanserin, (100 μg kg^{-1}; B).

The mean control (air-breathing), and plateau hypoxic discharge frequencies, mean slope (rate) of the dynamic phase of the response to hypoxia, and the time taken from application of hypoxia to steady state are shown in table 7.1 for data obtained before and after MDL, 10 μg kg^{-1} (n=6), MDL, 100 μg kg^{-1}, (n=12, in three of which MDL was injected after ketanserin), and ketanserin, 100 μg kg^{-1}, (n=5). Table 7.2 shows the mean values for PaO_2, $PaCO_2$, and pHa in the control and hypoxic states, before and after the injection of antagonists. No significant differences were detected (Wilcoxon two-sample test, two-tailed) in either the pre- and post- antagonist chemoreceptor responses, or blood gas analyses.

7.2.6 Effect of 5-HT in the rabbit.

5-HT (0.01-100 μg i.c.) was studied in one rabbit only, where it caused chemodepression, but no clear chemoexcitation, whether in the form of an initial transient, or as a delayed effect. Chemodepression was dose-related (Fig. 7.18), and the duration was from 6 to 20s. The ID_{50} for 5-HT evoked chemodepression was 10 μg, slightly higher than the mean value calculated for the same effect in cats. The effect of 5-HT injection upon blood pressure was a small transient fall, with the 50 and 100 μg doses only.

7.3 Summary of results presented in section 7.

1. Three separate components were identified in the complex response of carotid chemoreceptors to injected 5-HT. First, a transient burst of action potentials was obtained during the injection period in 56% of the recordings. Second, a period of dose-related chemodepression

Table 7.1: Effects of MDL 72222 and ketanserin on background (air-breathing) and hypoxic (10% O_2) discharge, time to plateau ('max') and rate of response to hypoxia.

	DISCHARGE (air; c.p.s.)	PLATEAU (10% O_2; c.p.s.)	TIME TO 'MAX' (s)	RATE (%max s^{-1})
Control (n=6)	6.8 ± 1.5	26.8 ± 5.3	112 ± 6	1.08 ± 0.11
MDL 72222 (10 µg kg^{-1}) (n=6)	10.8 ± 3.2	27.3 ± 5.8	109 ± 11	1.04 ± 0.12
Control (n=12)	5.3 ± 1.0	21.9 ± 3.2	114 ± 4	1.02 ± 0.07
MDL 72222 (100 µg kg^{-1}) (n=12)	7.7 ± 1.8	26.3 ± 5.5	116 ± 7	1.08 ± 0.11
Control (n=6)	6.9 ± 2.0	25.6 ± 6.5	112 ± 6	0.99 ± 0.06
Ketanserin (100 µg kg^{-1}) (n=5)	6.9 ± 2.0	26.0 ± 9.5	116 ± 17	1.02 ± 0.14

Table 7.2: Results of arterial blood gas analyses during normoxia (air-breathing) and hypoxia (10% O_2) before, and after the application of 5-HT antagonists.

	NORMOXIA	HYPOXIA	n
Control			
PaO_2	95.3 ± 2.4	36.2 ± 1.5	
$PaCO_2$	31.9 ± 1.1	31.1 ± 1.1	17
pHa	7.30 ± 0.01	7.32 ± 0.01	
MDL 72222, (10 µg kg^{-1})			
PaO_2	92.0 ± 7.0	35.5 ± 0.5	
$PaCO_2$	31.5 ± 0.5	30.5 ± 1.5	2
pHa	7.29 ± 0.04	7.32 ± 0.02	
MDL 72222, (100 µg kg^{-1})			
PaO_2	87.8 ± 1.7	39.5 ± 2.6	
$PaCO_2$	33.1 ± 0.9	31.5 ± 0.5	4
pHa	7.28 ± 0.02	7.33 ± 0.02	
Ketanserin, (100 µg kg^{-1})			
PaO_2	83.8 ± 7.7	34.1 ± 1.6	
$PaCO_2$	32.3 ± 2.3	33.3 ± 2.0	4
pHa	7.26 ± 0.05	7.28 ± 0.05	

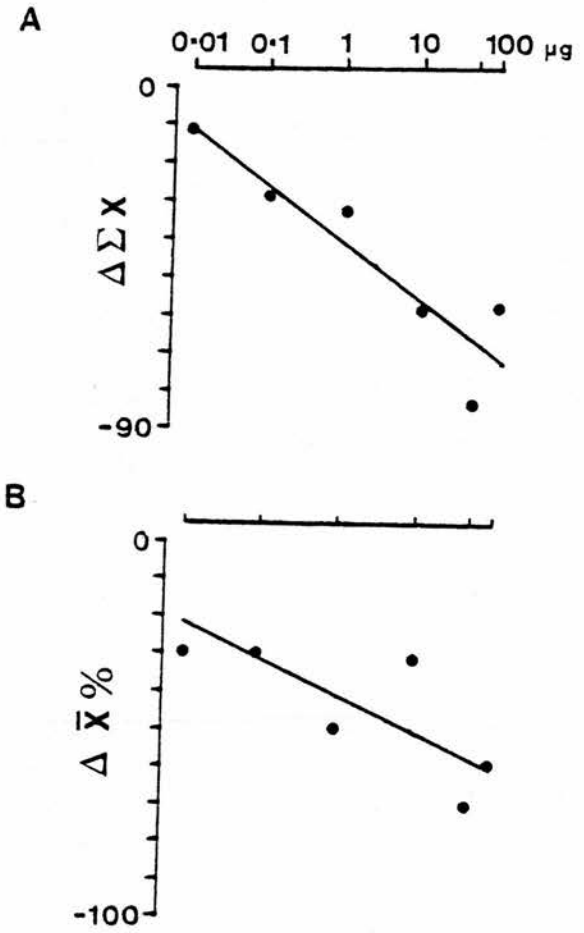


Figure 7.18. Dose-response data for the chemodepression evoked by 5-HT in a rabbit. (Transient chemoexcitation was not observed, and there was no clear secondary excitation). (A) $\Delta\Sigma x$ plotted against dose, and (B) $\Delta\bar{x}\%$ for the response occuring in the first five seconds after drug injections, plotted against dose. Lines were fitted to the data by the method of least squares.

commenced within a few seconds of completing the injection in all recordings, even in the absence of an initial transient excitation. Finally, a delayed non-dose-related but longer-lasting chemoexcitation occured in most experiments, simultaneous with a fall in systemic blood pressure.

2. The initial transient chemoexcitation was evoked by only one other 5-HT agonist, 2-Me 5-HT, which mimicked 5-HT in evoking all three components of the response, although the intensity of chemodepression alone was comparable to the effect seen with 5-HT itself. 5-MOT had virtually no effect upon chemoreceptor discharge. The 5-HT_1 agonists 8-OH DPAT and RU 24969, selective for the A and B sub-types respectively of this receptor had divergent effects, the former being a potent depressant of chemosensory discharge, and evoking little or no secondary excitation, whilst the latter was a weak depressor of chemoreceptors, but had potent excitatory effects.

3. The novel 'neuronal' 5-HT antagonist MDL 72222 virtually abolished the transient chemoexcitation evoked by 5-HT, and also significantly increased the mean ID_{50} for 5-HT-induced chemodepression, in a dose-related manner. In 37% of the recordings 5-HT caused dose-related chemoexcitation after the highest doses of the antagonist applied. Neither delayed chemoexcitation nor hypotension were obviously affected by the antagonist.

4. The 5-HT_2-selective antagonist ketanserin had no appreciable effect upon initial transient excitation in response to injected 5-HT, but there was a slight increase in the mean ID_{50} for 5-HT-evoked chemodepression. The delayed chemoexcitation and hypotension were both either abolished or substantially reduced by ketanserin.

5. All the effects of injected 5-HT upon chemoreceptor discharge

could be abolished by the combination of MDL and ketanserin, without the antagonists having any appreciable effect upon the capacity of chemoreceptors to increase their discharge frequency in response to hypoxic stimulation.

7.4 Discussion.

The present results show that 5-HT has complex, if somewhat variable, effects upon chemosensory discharge in anaesthetised cats. The limited data obtained in one rabbit do not suggest any marked difference from results in the cat, save that chemoexcitation was apparently lacking. Three separate components in the response to 5-HT were identified, and the antagonists MDL and ketanserin selectively affected different parts of the response. Earlier studies using respiratory reflexes as an index have provided evidence for both inhibitory and excitatory effects of 5-HT upon the carotid body chemoreceptors in various species, including the cat (Page, 1952; Douglas & Toh, 1953; Ginzel & Kottegoda, 1954), but 5-HT also induces complex and marked neurogenic and circulatory effects which are able to exert influences upon respiration independently of the chemoreceptors (Mott & Paintal, 1953; Comroe et al, 1953), making respiratory effects a rather poor index of chemoreceptor activity.

7.4.1 Transient chemoexcitation.

Transient chemoexcitation occured during the injection period in about half the recordings, and this effect has previously been described by Black et al (1972), Nishi (1975), and Docherty and McQueen (1978). The increase was only occasionally dose-related, which may be a consequence of tachyphylaxis. When identical doses of

5-HT are applied in close succession (e.g. less than 5 min appart - Nishi, 1975) either no response, or a much reduced effect is seen in response to the later doses; it should be noted that the effect more closely studied by Nishi, prone to tachyphylaxis, and considered by him to be the response to 5-HT is apparently the same as the transient chemoexcitation described here.

The rapid onset (i.e. during injection of 5-HT) suggests a direct action on the sensory nerve fibres (Eyzaguirre & Nishi, 1974). Whilst it cannot be denied that 5-HT could be acting indirectly, by release of endogenous substance(s) from, presumably, type I cells, to act upon the nerve endings, the time course of the response seems to preclude it from being secondary to 5-HT-induced vascular changes. The possible influences upon the chemoreceptors of increased sympathetic activity arising from the ganglion-stimulating action of 5-HT (e.g. Trendelenburg, 1958, 1959) were prevented by sectioning the ganglio-glomerular nerves. This cannot exclude the possibility that 5-HT may release stored NA from the terminals of sympathetic nerves that remain in situ in the carotid body, after transection of the nerve trunks. The finding that transient excitation was not obtained in all recordings, in agreement with Black et al (1972), could be due to differing sensitivities of myelinated and unmyelinated fibres to 5-HT, or perhaps to differences in the amount of 5-HT delivered by injection to the chemoreceptors whose activity is being recorded in any particular experiment. Tachyphylaxis cannot explain the absence of an excitatory response to the initial doses of 5-HT applied in an experiment, although in recordings where excitation did occur repeated administration could attenuate the response, possibly explaining the poor dose-dependency of the effect in some experiments.

Nishi (1975) considered this transient excitation to be the most consistent part of the response to 5-HT, and found it to occur with doses as low as 2 μg (i.c.); in the present experiments the effect was not clearly discernable at doses of less than 10 μg.

At the time of Nishi's studies LSD, methysergide, and gramine were widely used antagonists in the investigation of the effects of 5-HT upon peripheral and muscle 5-HT receptors (cf. Gyermek, 1966). LSD appeared to act as a partial agonist, since it caused chemoexcitation similar to 5-HT, but blocked only the vascular, and not the chemoreceptor effects of injected 5-HT (Nishi, 1975). In the same experiments gramine and methysergide also blocked the vascular effects of 5-HT, but not the chemoreceptor responses, nor did they themselves cause any chemoexcitation. This was taken as evidence that the 5-HT-evoked changes in chemoreceptor discharge are direct, rather than a consequence of vascular changes. Nishi was not able to classify the type of receptor responsible for excitation, although he did exclude direct or indirect involvement of nicotinc or muscarinic ACh receptors. Docherty and McQueen (1978) found that the DA antagonist α-flupenthixol at high doses could reduce the responses to 5-HT, but inconsistently. The 5-HT_2 antagonist ketanserin had no appreciable effect upon transient chemoexcitation, but the 'neuronal' antagonist MDL inhibited the effect.

MDL has been shown to have potent and selective blocking actions at excitatory 5-HT receptors located upon peripheral neurones (Fozard, 1984a). In particular, it antagonises responses to 5-HT mediated by receptors present upon the terminals of sympathetic fibres of the rabbit heart, at nanomolar concentrations; the actions of nicotinic agonists require much greater concentrations of the antagonist before they are inhibited. This compound also blocks the von Bezold-Jarisch

reflex evoked by 5-HT in the rat. It is only weakly active against responses mediated by the classic 'M' receptor (Gaddum & Picarelli, 1957) present upon cholinergic nerves of the guinea-pig ileum, nor does it block smooth muscle contractile responses mediated through 5-HT 'D' receptors, muscarinic or nicotinic ACh receptors, or histamine H_1-receptors, except at high concentrations. It appears that the bulk of the evidence points to the initial excitatory action of 5-HT upon chemoafferent fibres being the result of actions at a receptor, presumably within the carotid body, very closely associated with, or located upon the sensory nerve endings. Recent studies showing that MDL blocks the excitatory actions of 5-HT upon the cell bodies of rabbit vagal primary afferents (Azami et al, 1984) might be taken to support the hypothesis that 5-HT acts upon neuronal or sensory receptors in the carotid body.

The fact that MDL itself had certain 5-HT-like effects upon chemoreceptors could mean that the drug is a partial agonist. Of the other agonists studied, only 2Me 5-HT caused any transient chemoexcitation, but rather less potently than 5-HT itself.

7.4.2 Chemodepression.

5-HT caused a short-lasting chemodepression which commenced almost immediately upon completion of the injection, and which was dose-related in most experiments. Again, the rapid onset of the effect suggests that is not secondary to vascular changes caused by 5-HT. It was the most consistent component of the response, and occured regardless of whether or not the initial transient excitation was present. Depression of chemoreceptor discharge has also been previously reported (Black et al, 1972; Nishi, 1975; Docherty & McQueen,

1978). Earlier studies showed that very high doses of α-flupenthixol reduced the effect (Docherty and McQueen, 1978), but that a range of putative 5-HT antagonists did not alter this component of the response to 5-HT (Nishi, 1975). Nishi (1975) proposed that exogenous 5-HT induces depolarisation of the nerve endings, with very rapid onset, initiating nerve impulses at the non-myelinated portion of the sensory fibre, or at the first node; once depolarisation reaches a certain level, a cathodal block of the nerve occurs lasting several seconds. When depolarisation subsides, discharge reappears. This suggestion seems unlikely, for if this were the case, chemodepression should not occur in the absence of an initial depolarisation or excitatory effect, which is inconsistent with the present results. It is, of course, not impossible that 5-HT-induced depolarisation can be subthreshold, so far as generation of nerve impulses is concerned, but at the same time, sufficient to activate a hyperpolarising current, resulting in chemodepression in the absence of an obvious depolarisation.

Ketanserin had a rather variable effect upon 5-HT-induced chemodepression, usually causing a slight reduction, but sometimes a potentiation of the effect. In contrast, MDL caused a substantial dose-related antagonism of chemodepression, as shown by the increase in ID_{50} values, and higher doses could completely abolish the response - either unmasking an excitatory component, or potentiating the delayed secondary excitation.

The chemodepressant responses to 5-HT and to DA were compared before and after the antagonists domperidone, ketanserin, and MDL. Domperidone had no significant effect effect upon any phase of the chemoreceptor response to 5-HT when given in doses that substantially reduced DA-evoked chemodepression, and responses to DA were

unnaffected by either MDL or ketanserin. It may be concluded that chemodepression evoked by 5-HT does not involve a DA D_2-receptor, and is mainly mediated by mechanisms sensitive to the neuronal 5-HT antagonist MDL. The results with ketanserin might suggest that a small part of the chemodepression is attributable to actions upon 5-HT_2-receptors, assuming the antagonist is selective, and does not affect MDL-sensitive sites at the doses used. The possibility that depression of discharge results not from direct actions of 5-HT, but rather from the release of inhibitory substances (other than DA) cannot be ruled out, but the persistance of 5-HT-evoked chemodepression during naloxone infusion (see Section 8) tends to preclude the involvement of opioids, which are potent chemodepressant substances and are present in the carotid body.

Chemodepression was also elicited by 2Me 5-HT (an agonist at MDL-sensitive sites), but not by 5-MOT (which is relatively inactive at such sites). Of the 5-HT_1 agonists examined, a particularly marked chemodepressant effect was elicited by 8-OH DPAT, which is said to be selective for the A sub-type of 5-HT_1-receptors.

7.4.3 Delayed excitation.

The final component of the response to injected 5-HT was a delayed (10-30 s) phase of secondary excitation that lasted longer than any of the other components. It was rather variable, and, where present, its onset appeared to coincide with that of the fall in blood pressure caused by 5-HT. The antagonist MDL had no effect upon blood pressure, nor did it reduce the delayed chemoexcitation. Ketanserin inhibited both effects, and the reponses to injection of ketanserin itself may reflect some partial agonist properties of the

drug. It is not possible to say whether the delayed chemoexcitation was caused by the hypotensive effect, or resulted from actions upon 5-HT_2-receptors in the carotid body, more especially since onset of secondary excitation was much faster after the antagonist MDL, which did not alter the latency of the hypotensive effect. It is questionable how accurately changes in systemic blood pressure reflect vascular effects in the carotid body.

RU 24969 was a potent stimulant of chemoexcitation similar to the delayed effect seen after injection of 5-HT; other agonists tested caused little or no secondary excitation. It is perhaps a little anomalous that one of the 5-HT_1 agonists should cause an effect similar to that of 5-HT, apparently mediated through a 5-HT_2-receptor. More information concerning the biochemical events mediated by different 5-HT agonists at varying receptor sites is needed, and also the effects of this agonist after ketanserin must be studied, before any conclusions can be reached on this finding.

7.4.4 Classification of 5-HT receptors.

In the peripheral nervous system classification of 5-HT receptors is a complex problem. Amongst the effects mediated by these receptors are depolarisation, hyperpolarisation, and facilitation or depression of transmission. The net effect of 5-HT in the system under investigation may be excitatory or inhibitory, with little obvious relationship to the immediate effects of 5-HT/receptor interactions. With respect to sensory neurones, 5-HT may cause excitation or depolarisation of the cell body (Sampson & Jaffe, 1974; Higashi, 1977, Simonds & de Groat, 1980, Stansfeld & Wallis, 1981), or hyperpolarisation of the cell body (Higashi, 1977). Sensory axons may

be depolarised (Neto, 1978), whilst the nerve endings of various sensory fibres have been shown to be excited by 5-HT (e.g. Paintal, 1954 [gastric, but not pulmonary stretch fibres]; Fjällbrant & Iggo, 1961; Nishi, 1975).

In some respects, the effects of 5-HT upon sensory somata in the rabbit nodose ganglion (Higashi 1977) bear close resemblance to the chemoreceptor responses observed in these experiments, and the ionic currents that he determined could perhaps also underly the effects of 5-HT upon the chemoreceptors. In 8% of the neurones Higashi studied there was no response to 5-HT; 86% were, however, depolarised rapidly, and in a concentration-dependent manner by 5-HT, with concomitant repeated firing. Depolarisation was followed by an equally rapid repolarisation, and then an 'after'-hyperpolarisation of approximately 5 mV. This after-hyperpolarisation was in turn followed by a long-lasting depolarisation of a few millivolts. It was suggested that during the initial depolarisation response to 5-HT there is a simultaneous increase in the conductance of sodium and potassium ions, and during the after-hyperpolarisation only potassium ion-conductance remains increased. Both initial depolarisation and the after-hyperpolarisation were abolished in a sodium-free medium, which could mean that the hyperpolarising effect may be triggered by influx of sodium ions. There were indications that the residual depolarisation, which follows hyperpolarisation, is associated with a small increase in permeability to sodium ions. In about 6% of his recordings Higashi observed a simple hyperpolarisation (with neither initial, nor subsequent depolarisation), which was associated with a reduction in membrane resistance. Hyperpolarisation appeared to be caused by an increased potassium ion conductance, since it was enhanced by

removal of external potassium ions, and the polarity was reversed by displacing membrane potential beyond -90 mV.

Increased potassium and sodium ion conductances mediating the depolarisation of rabbit sympathetic ganglion cells has similarly been shown by Wallis and Woodward (1975) and Wallis and North (1978); after-hyperpolarisation, also observed by these authors, was attributed to activity of an electrogenic pump (cf. Lees & Wallis, 1975), activated by the influx of sodium ions.

Presumptive sensory myenteric neurones are usually hyperpolarised by 5-HT; the response is not subject to tachyphylaxis, and is accompanied by a fall in input resistance, possibly generated by an increase in potassium ion conductance (Johnson et al, 1980b).

In the light of these findings in other systems, it would be possible to ascribe all three components of the chemoreceptor response to injected 5-HT to sequential events following the activation of just one type of receptor, and this is empirically supported by the common blockade of transient chemoexcitation and chemodepression by the neuronal antagonist MDL.

Such a simplistic analysis may, however, not apply, since pronounced chemoexcitation, blocked by ketanserin, often appears after the application of MDL. The lack of effect of 5-MOT in causing any part of the typical response seen with 5-HT is encouraging, since the agonist is virtually inactive at MDL-sensitive sites (Fozard, 1984b). In contrast, 5-MOT is almost as effective an agonist as 5-HT at the classical 'M'-receptor. Since 5-MOT causes no marked changes in chemoreceptor activity, this may exclude 'M'-receptors from the mediation of the 5-HT response, hence, perhaps, Nishi's failure to classify the 'chemoreceptor' 5-HT-receptors, using

conventional 5-HT antagonists.

The basis of classifying certain 5-HT receptors as 5-HT_1 or 5-HT_2 derives from the differential binding of tritiated ([^{3}H]-) 5-HT and spiroperidol (SPIR) to two different populations of post-synaptic receptors located on membranes of brain tissue obtained from different locations. [^{3}H]-LSD appears to bind to both types of receptor with equal affinity (Peroutka & Snyder, 1979). Morphine is inactive at both receptor subtypes, and phenoxybenzamine only weakly so. Thus, neither fits the scheme of 'M' (morphine sensitive) or 'D' (sensitive to phenoxybenzamine-like drugs) proposed by Gaddum and Picarelli (1957). The potencies of various agonists and antagonists differ widely in competing for [^{3}H]-5-HT (5-HT_1) and [^{3}H]-SPIR (5-HT_2) binding. [^{3}H]-5-HT binding has been shown to be inhibited by guanine nucleotides, in a manner comparable to the regulation of receptor binding of neurotransmitters whose effects are linked to an adenylate cyclase (Peroutka et al, 1979). This suggests, at least indirectly, that 5-HT_1 receptors may exert their effects via cAMP. 5-HT-sensitive adenylate and guanylate cyclases have been identified in the CNS (Pagel et al, 1976; Quayle et al, 1978), but not in the peripheral nervous system (Wallis, 1981).

There is ample evidence (Bennett & Snyder, 1976; Fillion et al, 1976; Leysen & Laduron, 1977; Leysen et al, 1978; Nelson et al, 1978) that 5-HT_1 and 5-HT_2 receptors are different molecular entities. Ketanserin has a high affinity for 5-HT_2-receptors (Leysen et al, 1981; Leysen et al, 1984), and ketanserin-sensitive sites appear to be involved in central mediation of certain behavioural activities. In isolated tissues they can mediate vasoconstriction, tracheal smooth

muscle contraction, as well as bronchoconstriction in vivo (Leysen et al, 1984). Activation of platelets by 5-HT, particularly in the cat, appears to be mediated via 5-HT_2-receptors (de Clerck et al, 1984, de Clerck & Herman, 1983; Leysen et al, 1983). It is thought that binding to 5-HT_2 receptors activates phoshpolipase C, causing formation of diacylglycerol (Leysen et al, 1984). Signal transmission through increased turnover of phospholipid involves release of calcium ions from membrane-chelated stores to activate phospholipase C (Erne et al, 1983), leading to generation of diacylglycerol - which is a potent activator of protein kinase C. Activation of protein kinase C has been proposed as the key event in the transmission of receptor stimuli (Nishizuka, 1983), and has an absolute requirement for calcium ions and phospholipid, although its target proteins in most tissues remain to be clarified. Such mechanisms presumably require a finite amount of time in which to become active, and the 5-HT_2-receptor may be capable of subserving the secondary excitation of chemoreceptors that can be blocked by ketanserin. It may be fortuitous that, in the absence of MDL, this part of the response appears to coincide with the hypotensive response to 5-HT, for ketanserin is inactive at receptors mediating relaxation of contracted vessels (van Nueten et al, 1984). Blockade of MDL-sensitive receptors may result in a significantly larger quantity of the injected 5-HT being available for binding with chemoreceptor 5-HT_2-receptors (if such exist) than when the MDL-sensitive sites are free to bind the drug. The possibility that there is a balance between the effects mediated by MDL- and ketanserin- sensitive sites needs to be resolved.

The effects of 5-MOT and 2Me 5-HT upon chemoreceptors are

consistent with the hypothesis that transient chemoexcitation and chemodepression are the major effects of exogenous 5-HT, and that they are both mediated via MDL-sensitive sites. Since only slight secondary excitation was evoked by 2Me 5-HT, there is still the possibility that this part of the response is mediated by a receptor different to that or those mediating the earlier parts of the response. Although 5-HT_2-receptors are implicated in the mediation of delayed excitation, RU 24969-sensitive 5-HT_{1B}-receptors may also be involved, because of the potent effects of this agonist. It is not possible to say whether or not this excitation involves the same receptors as those activated by 5-HT.

Controversy still surrounds the classification of 5-HT receptors (cf. Wallis, 1981; Fozard, 1984b; Humphrey, 1984), and the rather perplexing array of effects of 'selective' 5-HT agonists upon chemoreceptor activity will only be resolved when further information, in particular concerning the biochemical events mediated by these agonists at different receptors, is forthcoming.

7.4.5 Physiological Stimulation.

Although the combination of MDL and ketanserin effectively blocked the actions of injected 5-HT upon chemosensory discharge, the response of chemoreceptors to physiological stimulation by hypoxia was unaltered. Assuming the antagonists reached effective concentrations at sites within the carotid body where locally-released 5-HT might act, the implication is that endogenous 5-HT has no vital role in the mechanism of chemoreception. However, the possibility that 5-HT might exert more subtle influences must be borne in mind, especially in view of its reported co-storage with substance P in neurones (see, for

example, Hökfelt et al, 1980), and the potentiation of its effects during SP infusion (see Section 9).

Reports of the conditions under which 5-HT is released in the carotid body, if indeed it is, are still awaited. Histochemical demonstration of 5-HT in type I cells clustered around blood vessels in the carotid body of the rat might point to some function as a vasoactive agent, and in this respect it is interesting that increased levels of 5-HT have been reported in the carotid bodies of hypertensive subjects, where an abnormal blood flow might necessitate some compensatory mechanism to maintain normal chemoreception.

SECTION 8

THE EFFECTS OF OPIOID PEPTIDES UPON CHEMOSENSORY ACTIVITY IN THE CAT

SECTION 8.

THE EFFECTS OF OPIOID PEPTIDES UPON CHEMOSENSORY ACTIVITY IN THE CAT.

8.1 Introduction.

Interest in the enkephalins as possible transmitters or neuromodulators in the carotid body stems from the discovery that [Met]enk and [Leu]enk are present in the carotid body (e.g. Wharton *et al*, 1980), and are possibly stored with NA in type I cells (Hansen *et al*, 1982). When applied *in vivo* or *in vitro* they are able to alter chemoreceptor activity. Monti-Bloch and Eyzaguirre (1980b) reported that [Met]enk increases chemoreceptor discharge *in vitro*, but McQueen and Ribeiro (1980) showed that it is a potent depressant of chemosensory activity in the cat *in vivo*, the effect being at least partially blocked by the opioid antagonist naloxone (NAL - McQueen & Ribeiro, 1980, 1981b). NAL itself appears to have only slight effects upon chemoreceptor discharge *in vivo* (McQueen & Ribeiro, 1980), but the fact that the response to hypoxia may be enhanced after NAL (Pokorski & Lahiri, 1981) does suggest that opioids (i.e. enkephalins) may exert some tonic inhibitory action.

Related substances such as morphine and β-endorphin (β-END) have only weak (excitatory or depressant) effects upon chemoreceptor discharge *in vivo*, and these are blocked by NAL (McQueen & Ribeiro, 1980, 1981b). ENK-mediated chemodepression is likely to be mediated through specific activation of opioid receptors because of the antagonism by NAL, and also the failure of α-flupenthixol to block the effect at doses which abolish DA-evoked chemodepression (McQueen, 1981); similarly, the effect of DA is unaltered by NAL (McQueen &

Ribeiro, 1980).

In contrast, the antinociceptive effects of enkephalins are weaker than those of morphine; although both the enkephalins and morphine display similar potencies in receptor binding assays, the doses of ENK required for an analgesic effect are generally 50-100 times greater than those of morphine, and must be administered specifically to the cerebral ventricles, cisterna magna or periaqueductal grey matter. Even then the effect is weak and short-lived (cf. Schwartz et al, 1981). The in vivo potency of enkephalins is greatly increased in analogues rendered less susceptible to degradation by peptidases (see Schwartz et al, 1981). Replacement of the L-Gly^2 residue by a D-amino acid (e.g. D-Ala, D-Met or D-Thr) and/or N-methylation of the Tyr^1 residue increases potency in vivo (but does not alter in vitro receptor binding properties). An increase in potency is also achieved in compounds containing an N-methyl-Phe^4 residue, a D-amino acid in position 5, or in which the terminal carboxyl group is acidified, esterified or replaced by an alcohol (Morley, 1980).

Differing pharmacological profiles of a number of opiate drugs and neuropharmacological or behavioural tests in spinal dogs led to the proposal of three different receptor sub-types - μ (morphine-sensitive), κ (ketazocine-sensitive), and σ (N-allylnormetazocine (SK&F 10047)-sensitive - see Gilbert & Martin, 1976; Martin et al, 1976). Another receptor type was identified and designated δ in the mouse vas deferens, this receptor being different from the μ- and κ- sites, and with which the enkephalins appear to interact preferentially (Lord et al, 1977); the antagonistic effects of NAL at this receptor were also observed to be less potent than its actions at μ-receptors.

Most of the opioid or related drugs used in this study (see

Appendix 2) were chosen because of their increased resistance to peptidase degradation (which enhances their potency) and because of their reported greater (though by no means absolute) selectivity at differing receptor sub-types. This study was performed to determine which opioid receptor sub-types might mediate the ENK effects upon chemoreceptor activity, and to determine whether any particular opioid would be more appropriate for use in the investigation of the interaction of opioids and substances such as NA (with which there appears to be co-storage in the carotid body - see above - and in the adrenal medulla - cf. Viveros et al 1980), DA, ACh or 5-HT. [Met]enk and [Leu]enk are typically δ-receptor ligands; [D-Ala^2, D- Leu^5]enk (DADL) is a commonly employed δ-'selective' agonist, although it is only twelve times more active at the δ-site than at μ-receptors (Paterson et al, 1983). [D-Ala^2, MePhe^4, Gly- ol^5]enk (DAGO) is some 220 times more active at μ- than at δ- binding sites (Kosterlitz & Paterson, 1981); the casein-derivative morphiceptin (MCPT; Chang et al, 1981) is also selective for μ- receptors, but less potent. Benzomorphans, ketazocine-like compounds and dynorphins have been shown to exhibit preferential activity at κ- receptors (e.g. Gilbert & Martin 1976; Martin et al, 1976; Corbett et al, 1982), and the compounds ethylketocyclazocine (ETZ) and dynorphin octapeptide (dynorphin $_{(1-8)}$ - DYN) were used in this study to determine the possible contribution of κ-receptor activation to the response of chemoreceptors to opioids.

NAL-blockade of the chemodepressant effect of enkephalins is incomplete (see above) and its specificity, particularly at high doses has been questioned (Sawynok et al, 1979). The antagonists used in this study (see Appendix 2) were the more recently developed substituted enkephalins ICI 154,129, which is more selective for δ-receptors

(Shaw et al, 1982) and ICI 174,864 which is also selective for δ-receptors, but more potent than the former antagonist (Cotton et al, 1984).

8.2 Methods.

The effects of i.c. injection of a range of doses of agonists were studied before and after different doses of antagonists. In three experiments hypoxia tests (see Sections 2 and 4) were carried out during infusions of [Met]enk and the effects of raising the inspired CO_2 to 5% were studied in the same experiments. Comparison was made with the same tests performed during infusion of CO_2-air-equilibrated Locke solution. To study the interactions of monoamines and ACh with enkephalins these drugs were injected at 90 seconds of a two-minute infusion of [Met]enk, the amount of opioid infused being 2 or 10 μg min^{-1}, sufficient to cause an obvious depression of chemoreceptor discharge, the dose varying in different experiments depending upon the sensitivity to [Met]enk. Short infusions were prefered so as not to desensitise the preparation. Because of the lack of material the more selective antagonists could not be used in the 'interactions' experiments; instead NAL was infused at a level of 600 μg min^{-1} (determined by experiment) for two minutes, with injections of monoamines being made at 90 seconds of the infusion. In all experiments controls were carried out by making injections of monoamines during infusion of Locke solution (0.1 ml min^{-1}), and in the case of NAL-infusions single injections of [Met]enk (e.g. 1 μg i.c.) were made to determine the effectiveness of opioid antagonism.

8.3 Results.

8.3.1 Dose-response data.

[Met]enk is the most abundant opioid peptide found in the carotid body of the cat (Wharton et al, 1980), and was used as the 'reference' compound. When injected i.c. [Met]enk caused chemodepression in all experiments (cf. Figs. 8.3, 8.6). Dose response data were obtained in thirteen cats; depression of discharge (which was usually 'absolute' for 5-15 s) was dose-related in all cases (e.g. Fig. 8.1). Following the initial depression of discharge, with doses of 1 μg or greater, the rate of discharge gradually increased back to control levels, with the total period during which discharge frequency was reduced lasting for a minute or more. It was determined that the optimum method of quantifying this effect was to calculate the mean discharge frequency in the first 30 s after injection and to express this as a percentage change from the appropriate pre-injection control ($\Delta\bar{x}\%$ - see Fig. 8.1). If a shorter time interval was chosen (e.g. 5 s, cf. analysis of 5-HT effects, Section 7), a 'maximum' response could be demonstrated with very low doses of agonist; the effects of control injections of Locke solution appeared to be inordinately large when compared to the effects of the peptide on this time scale. When mean discharge was calculated in a longer time period (e.g. 1-2 mins) the effects of low doses of the drug were largely nullified. Subsequently, this method of analysis was applied for all opioid-like drugs used, to enable comparison of dose-response data. From dose-response curves such as that illustrated in Fig. 8.1 the ID_{50} (dose causing a 50% decrease in discharge from pre-injection control discharge, in the first 30 s after injection) values were determined, and are presented in table

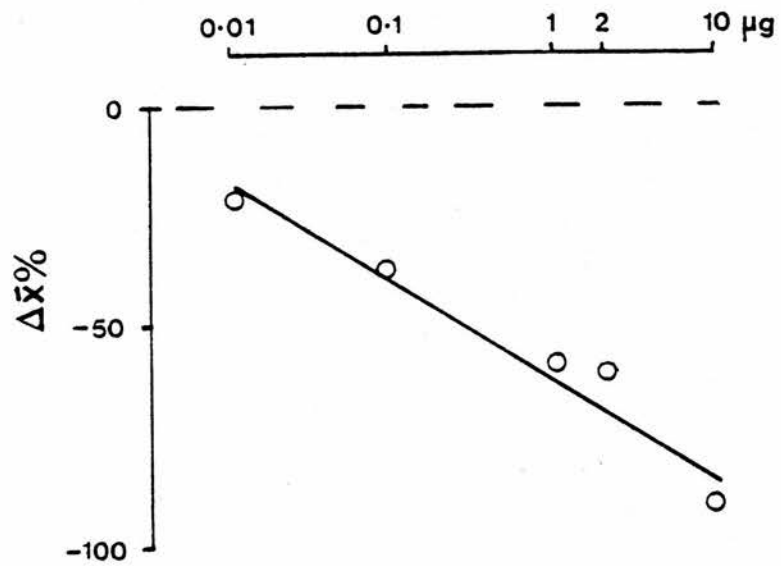


Figure 8.1. Dose-response curve for [Met]enk-evoked chemodepression (in a single experiment) showing the relationship between dose and the average discharge in the first thirty seconds after injection expressed as a percentage change from pre-injection control. The straight line was fitted to the data using the method of least squares.

Table 8.1: Mean ID_{50} values (± s.e.m.) calculated for the range of opioid agonists.

Agonist	Selectivity	Dose range (μg)	ID_{50} (μg)	ID_{50} (nmol)	n
[Met]enk	δ	0.001-100	0.46 ± 0.07	0.79 ± 0.13	13
[Leu]enk	δ	0.001-100	1.44 ± 0.80	2.60 ± 1.45	5
DADL	δ	0.001-100	1.23 ± 0.74	2.16 ± 1.30	3
DAGO	μ	0.001-100	9.0 ± 2.4	17.5 ± 4.7	5
DYN	κ	0.01-100	47.32 ± 15.56	48.24 ± 15.86	5
EKZ	κ	0.01-100	966 ± 545	2399 ± 1353	5
MCPT	μ	0.01-100	(No chemodepression evoked by this compound)		

8.1. An example of dose-response curves, obtained in the same experiment, for a δ-agonist ([Met]enk) and for a κ-agonist (DYN) is shown in Fig. 8.2. From the pooled data it was determined that the rank order of potency as depressors of chemoreceptor activity (cf. table 8.1) was: [Met]enk ≥ [Leu]enk = DADL > DAGO >> DYN >> EKZ >>> MCPT = 0.

ID_{50} values calculated for δ-'selective' agonists were significantly lower (Student's t-test) than those calculated for the other agonists. MCPT did not cause any appreciable depression of discharge but, instead, an approximately 20% increase in discharge at all doses in the single experiment where studied.

All agonists caused some degree of change in systemic arterial blood pressure, the observed effect being hypotension at high doses (greater, generally, than the value of ID_{50}).

8.3.2 Effects of novel selective antagonists.

ICI 154129 (0.1-10 mg kg^{-1} i.c.) was studied in seven experiments. The antagonist blocked [Met]enk-evoked chemodepression (Fig. 8.3) and caused the dose-response curve for chemodepression to shift upwards and to the right (Fig. 8.4); the mean ID_{50} was significantly increased ($P<0.05$ - Student's t-test) in a dose-related manner (Table 8.2) after 1 and 10 mg kg^{-1} doses of the antagonist. Fig. 8.5 shows the effect of the antagonist upon ID_{50}; despite variability, ID_{50} was clearly increased by the lowest dose used (in all but one of three where tested experiments), and increasing the dose of antagonist caused the ID_{50} value to be raised still further.

The effects of ICI 174864 were examined in four experiments, where the chemodepressant effects of [Met]enk were blocked by

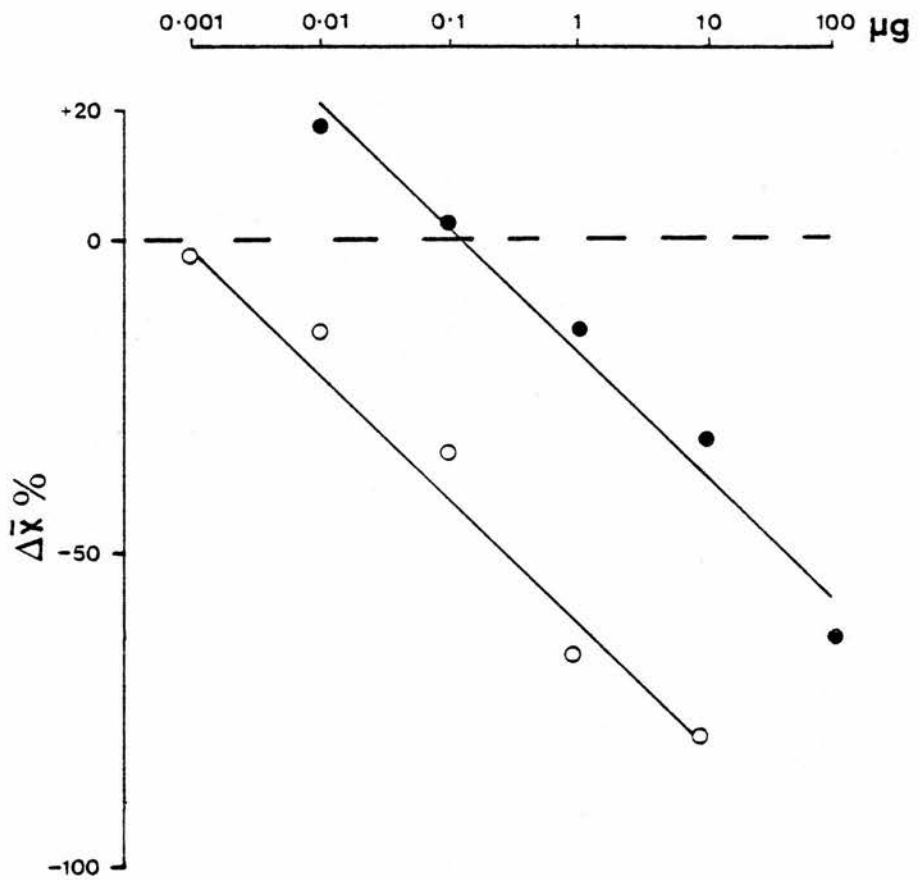


Figure 8.2. Comparison of the dose-response curves for chemodepression evoked by the 'δ'-agonist [Met]enk and the 'κ'-agonist DYN in the same experiment. Average discharge in the first thirty seconds after injection, expressed as a percentage change from pre-injection control, has been plotted against dose. The straight lines were fitted to the data using the method of least squares.

Data for responses to [Met]enk and DYN are denoted by open and closed symbols respectively.

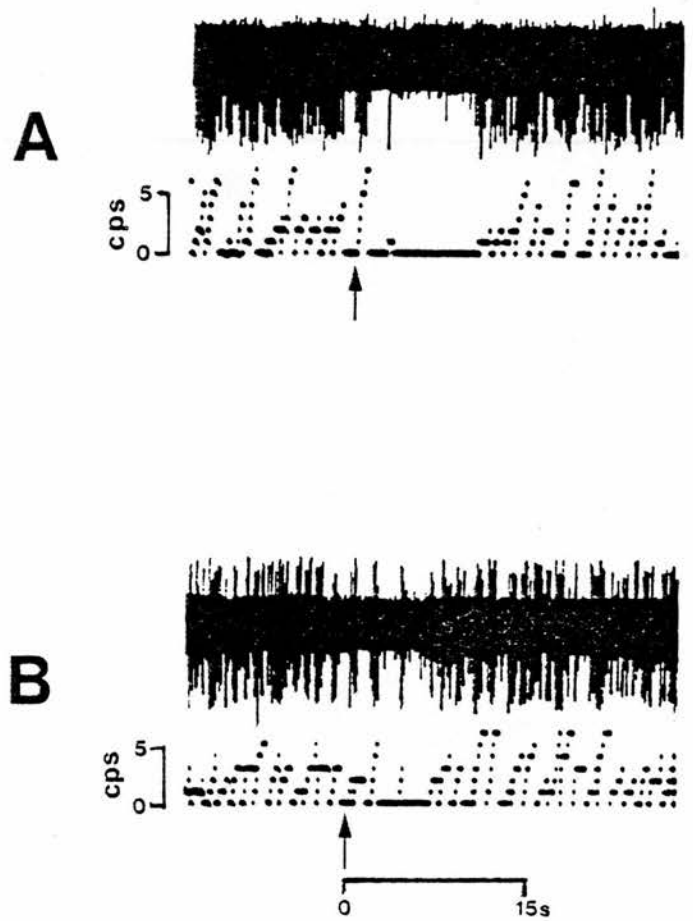


Figure 8.3. Neurogram showing the response of 3-4 chemoreceptor units to the injection of [Met]enk (0.1 μg i.c. at arrows) before (A) and after (B) injection of the antagonist ICI 154129, 10 mg kg^{-1} i.c. The discriminator was set to count just two units, and the ramped counter output beneath each neurogram shows the number of action potentials counted in successive 1 s intervals. The depression of discharge by [Met]enk and antagonism of the effect by ICI 154129 are both clearly displayed.

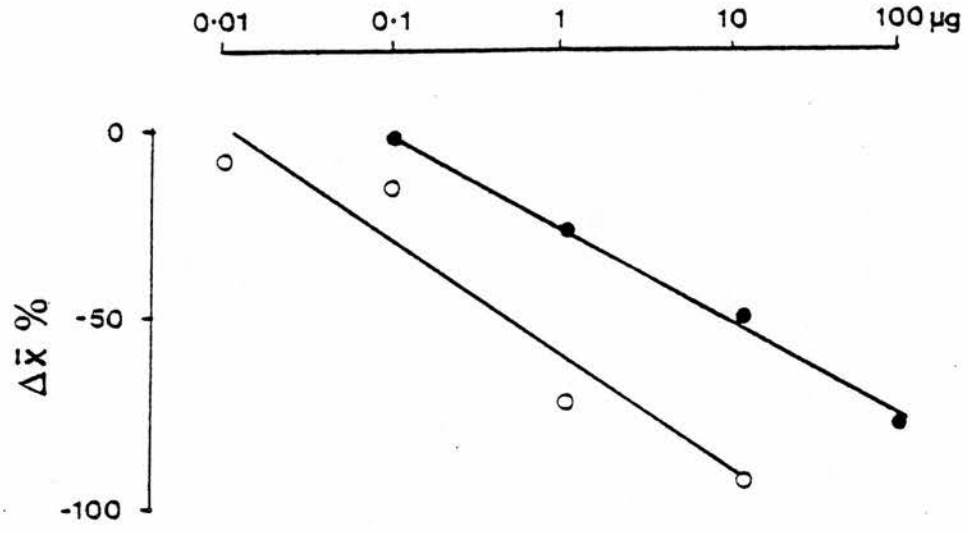


Figure 8.4. Comparison of the dose-response curves for chemodepression evoked by [Met]enk before and after ICI 154129, 10 mg kg^{-1} i.c. in one experiment. Average discharge in the first thirty seconds after injection, expressed as a percentage change from pre-injection control, has been plotted against dose. The straight lines were fitted to the data using the method of least squares.

Data for responses to [Met]enk obtained before and after ICI 154129 are denoted by open and closed symbols respectively.

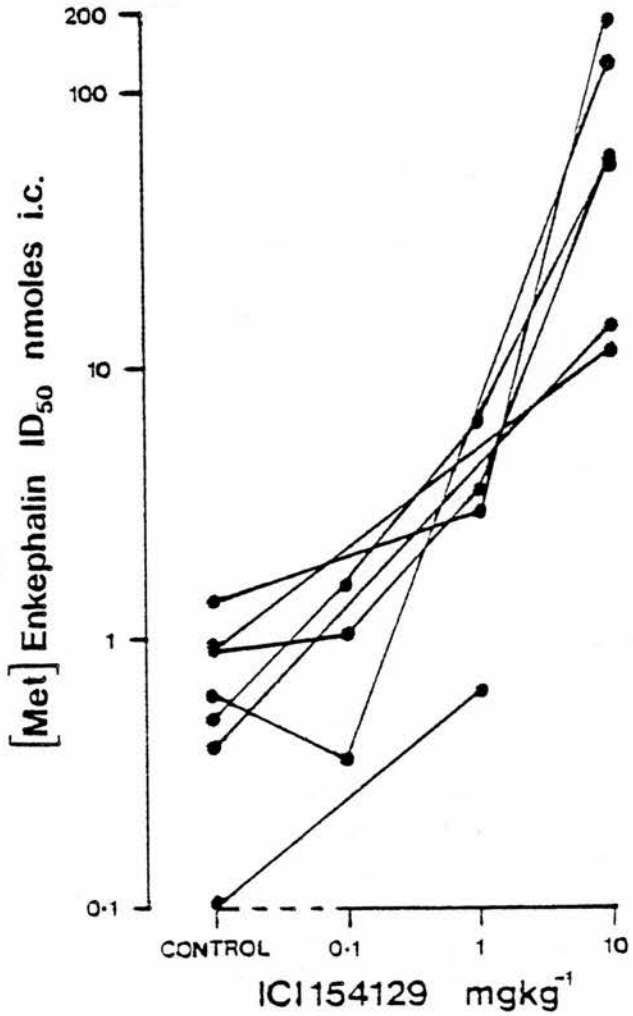


Figure 8.5. Comparison of ID_{50} values for [Met]enk-evoked chemodepression obtained before (control) and after increasing doses of the antagonist ICI 154129 in seven different experiments, plotted on the same axes. The increase in ID_{50}, reflecting antagonism of the chemodepressant effect of [Met]enk, is clearly dose-related in all experiments. Note that the scales of both axes are logarithmic.

Table 8.2: Mean ID_{50} values (± s.e.m.) for [Met]enk-evoked chemodepression calculated before and after increasing doses of the antagonist ICI 154129.

	ID_{50} (µg)	n
Control	0.46 ± 0.07	13
ICI 154129 0.1 mg kg^{-1}	0.58 ± 0.22	3
ICI 154129 1.0 mg kg^{-1}	2.48 ± 0.74*	4
ICI 154129 10.0 mg kg^{-1}	41.62 ± 15.04*	6

* $P<0.05$ with respect to control (Student's t-test).

comparatively low doses of the antagonist (Figs 8.6, 8.7). The mean ID_{50} for [Met]enk-evoked chemodepression in these four experiments was 0.35 ± 0.1 μg (0.62 ± 0.2 nmol). After ICI 174864 0.1 mg kg^{-1} (n=2) the ID_{50} was 3.75 ± 0.05 μg (6.5 ± 0.1 nmol), and after the higher dose of antagonist, 0.2 mg kg^{-1} (n=3), the ID_{50} increased still more to 132 ± 67.3 μg (231 ± 117 nmol). These differences were significant (Wilcoxon ranked test), and when presented as in Fig. 8.8 it is clear that there was a marked and potent antagonism of the effect of [Met]enk in all experiments. It should be noted that the highest dose of this antagonist was approximately fifty times smaller than that of ICI 154129.

Chemodepression evoked by [Leu]enk was markedly reduced after ICI 174864; after the lower dose of antagonist ID_{50} increased two thousand-fold in one experiment, and in a separate experiment the increase in ID_{50} after the higher dose of antagonist was increased by a factor of ten thousand. DADL and DAGO were also studied after ICI 174864, although data for full dose-response curves after the antagonist were not obtained. Chemodepression evoked by single or a few doses of these agonists was markedly attenuated in all four experiments, and there was some evidence of an excitatory effect replacing chemodepression, after the antagonist.

Both ICI 154129 and ICI 174864 had only slight effects upon basal rate of discharge; there was a slight but significant increase ($P<0.05$; Wilcoxon two sample test) in one out of four experiments, after ICI 154129. After ICI 174864 (n=4) discharge was significantly increased in one experiment ($P<0.05$; Wilcoxon test) and only slightly increased in one, and decreased in the remaining two experiments.

ICI 154129 partially blocked the hypotensive effect of [Met]enk,

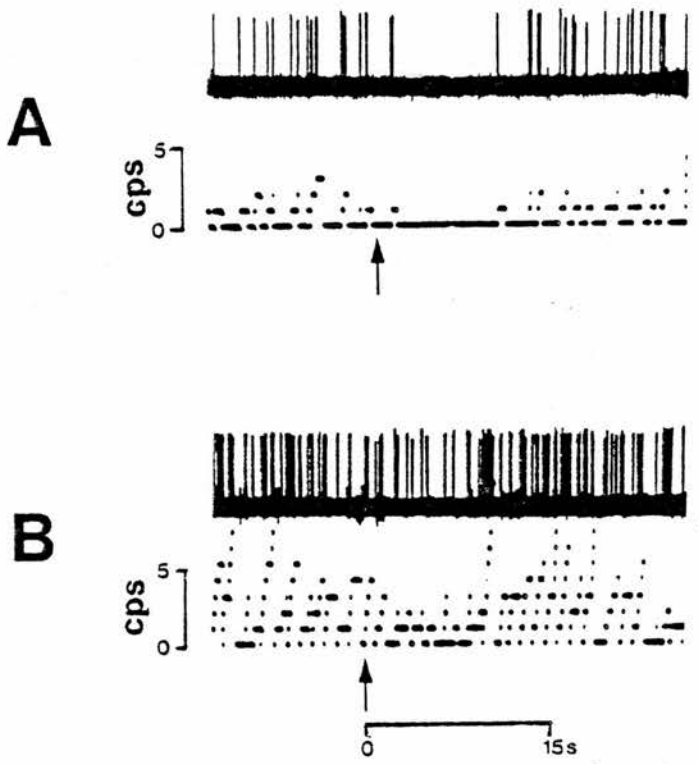


Figure 8.6. Neurogram showing the response of a single chemoreceptor unit to the injection of [Met]enk (0.1 µg i.c. at arrows) before (A) and after (B) injection of the antagonist ICI 174864, 0.2 mg kg^{-1} i.c. The ramped counter output beneath each neurogram shows the number of action potentials counted in successive 1 s intervals. Opioid-evoked chemodepression and antagonism of the effect are both clearly shown.

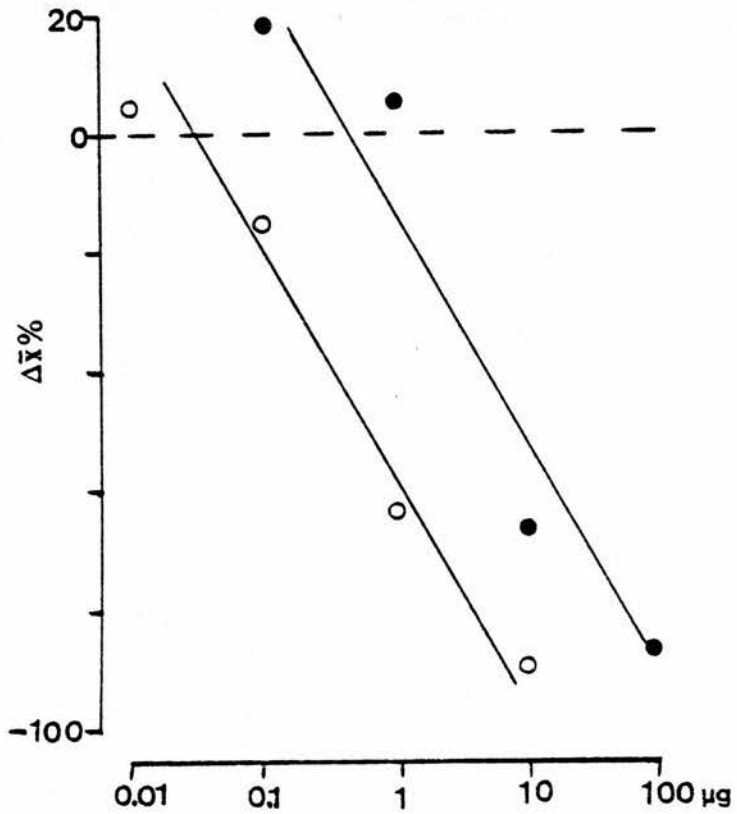


Figure 8.7. Comparison of the dose-response curves for chemodepression evoked by [Met]enk before and after ICI 174864, 0.2 mg kg^{-1} i.c., in one experiment. Average discharge in the first thirty seconds after injection, expressed as a percentage change from pre-injection control, has been plotted against dose. The straight lines were fitted to the data using the method of least squares.

Data for responses to [Met]enk obtained before and after ICI 174864 are denoted by open and closed symbols respectively.

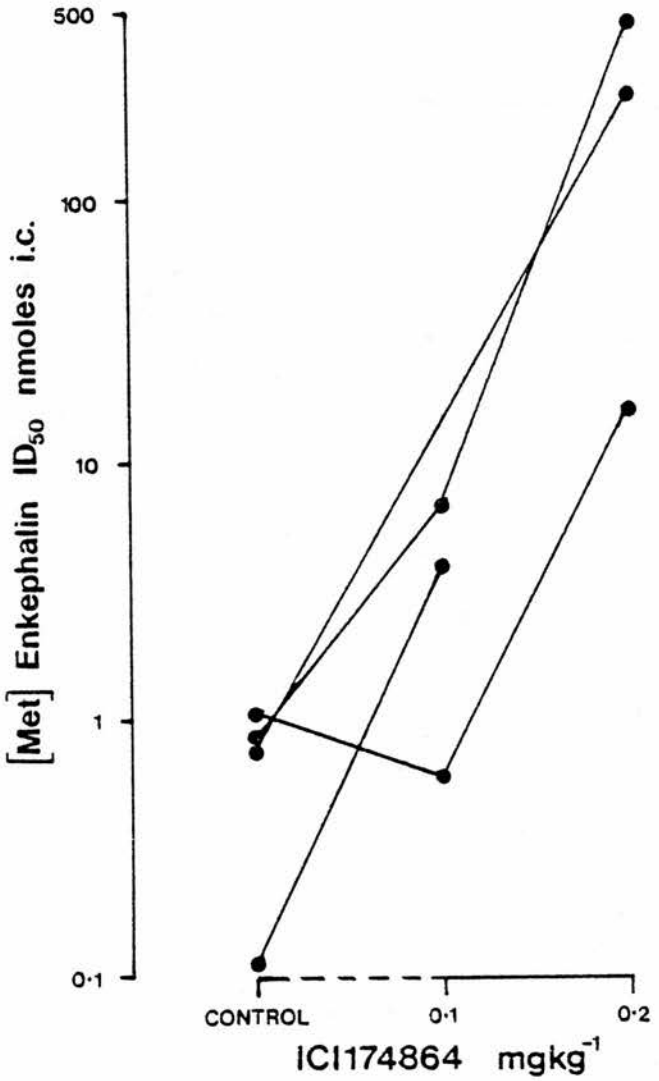


Figure 8.8. Comparison of ID_{50} values for [Met]enk-evoked chemodepression obtained before (control) and after different doses of the antagonist ICI 174864 in four different experiments, plotted on the same axes. The increase in ID_{50}, reflecting antagonism of the chemodepressant effect of [Met]enk, is clearly dose-related in all experiments. Note that the scales of both axes are logarithmic.

but the antagonist ICI 174864 had no obvious effect upon opioid-induced changes in systemic arterial blood pressure.

Because of the apparently greater sensitivity of chemoreceptors to [Met]enk, and to allow possible comparison with other studies (see introduction) this opioid was used in the remaining parts of the experiments.

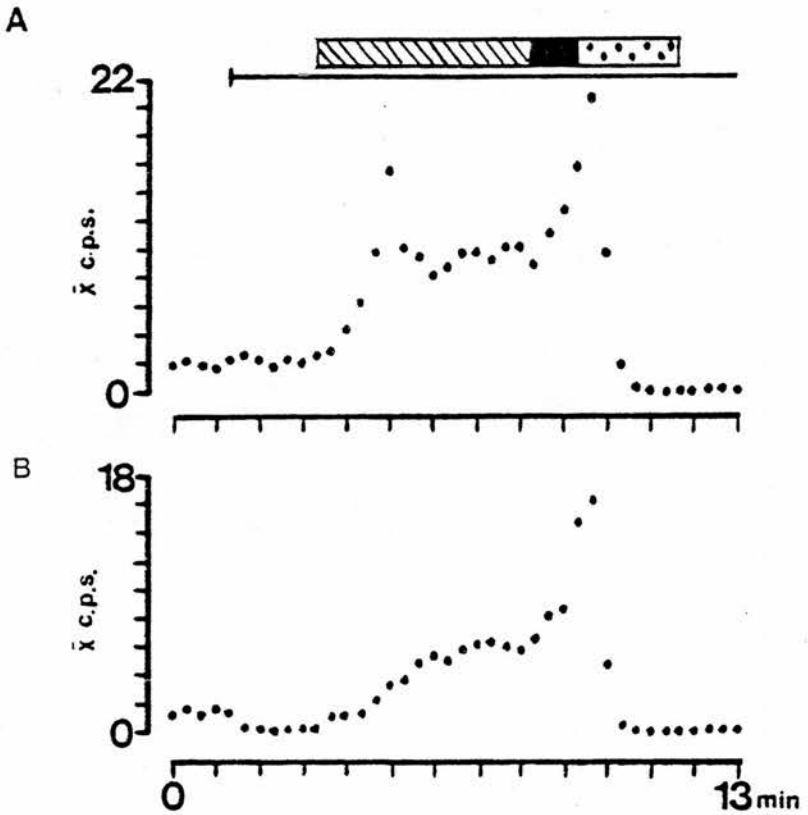
8.3.3 Interactions with hypoxia or hypercapnia.

Hypoxia tests were performed during infusion of [Met]enk (2 μg min^{-1}, n=1 or 10 μg min^{-1}, n=2) in three cats. Data were analysed as described in sections 2, 4, and 5. [Met]enk infused at either concentration depressed the basal rate of discharge (Fig. 8.9) and markedly attenuated the response to hypoxia, without abolishing it (Fig. 8.9). Rate of increase in chemoreceptor discharge in response to 10% O_2-hypoxia was not significantly altered (see table 8.3). Because of the variability between experiments mean chemoreceptor discharge frequencies were not significantly different at any stage of the test when data obtained during [Met]enk or Locke infusion were compared (table 8.3). When discharge on 10% O_2 during Locke infusion, in each experiment, was set as 100%, and discharge levels related to this, then rate of discharge at all stages of the hypoxia test was significantly reduced during infusion of [Met]enk (table 8.3).

Chemoreceptor discharge increased when animals were ventilated with 5% CO_2, whilst PaO_2 was maintained as near constant as experimental conditions allowed. This test was performed in three cats and [Met]enk infusion (2 μg min^{-1}, n=1, or 10 μg min^{-1}, n=2) depressed the activity of chemoreceptors during normocapnia and during hypercapnia (Fig. 8.10). The responsiveness of chemoreceptors to CO_2

Figure 8.9. (A) Responses to hypoxia (hatched bar, 10% O_2; solid bar, 100% N_2; stippled bar, 100% O_2) during infusion (horizontal bar) of Locke solution (0.1 ml min^{-1}) and (B) [Met]enk 2 μg min^{-1} in a single experiment.

(C,D,E): hypoxia responses in three different experiments during infusion of Locke solution (0.1 ml min^{-1}; open symbols) and [Met]enk (2 or 10 μg min^{-1}; closed symbols). 'Plateau' discharge during 10% O_2-hypoxia whilst infusing Locke solution has been set at 100% in each experiment, and the levels of discharge during ventilation with air, 10% O_2, and 100% N_2 have been related to this degree of chemoreceptor activity. The opioid depressed chemoreceptor discharge at all stages of the test, in each experiment.


A
22
x̄ c.p.s.
0
B
18
x̄ c.p.s.
0
0
13 min

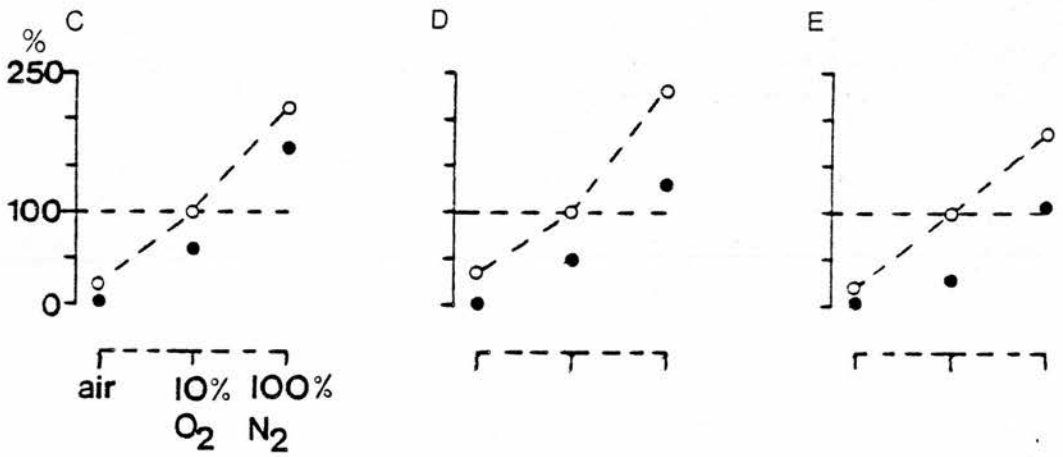

C
%
250
100
0
air
10% O2
100% N2
D
E

Table 8.3: Effects of [Met]enk infusion (2-10 μg min^{-1}) upon parameters defining the responsiveness of chemoreceptors to hypoxia.

	Locke inf. (0.1 ml min^{-1})	[Met]enk inf.	mean difference (± s.e.m.)	n
Control $\bar{x}$ c.p.s.	4.6	0.5	4.22 ± 2.2	3
Plateau $\bar{x}$ c.p.s.	17.0	7.7	9.4 ± 3.2	3
N_2 peak $\bar{x}$ c.p.s.	36.9	22.5	14.4 ± 7.1	3
Slope (% max s^{-1})	0.62	0.34	0.29 ± 0.09	3
Time to plateau (s)	151	157	5.7 ± 25.8	3
Control c.p.s. %	25.1	3.1	21.9 ± 4.7*	3
Plateau c.p.s. %	100	46	54.0 ± 9.8*	3
N_2 peak c.p.s. %	211	136	75 ± 16.5*	3
PaO_2 (10% O_2; mm Hg)	39.3	45.0	5.7 ± 3.2	3
$PaCO_2$ (mm Hg)	32.2	31.2	1.0 ± 3.9	3

* $P<0.05$ ([Met]enk results compared with Locke-infusion results - Student's paired t-test.)

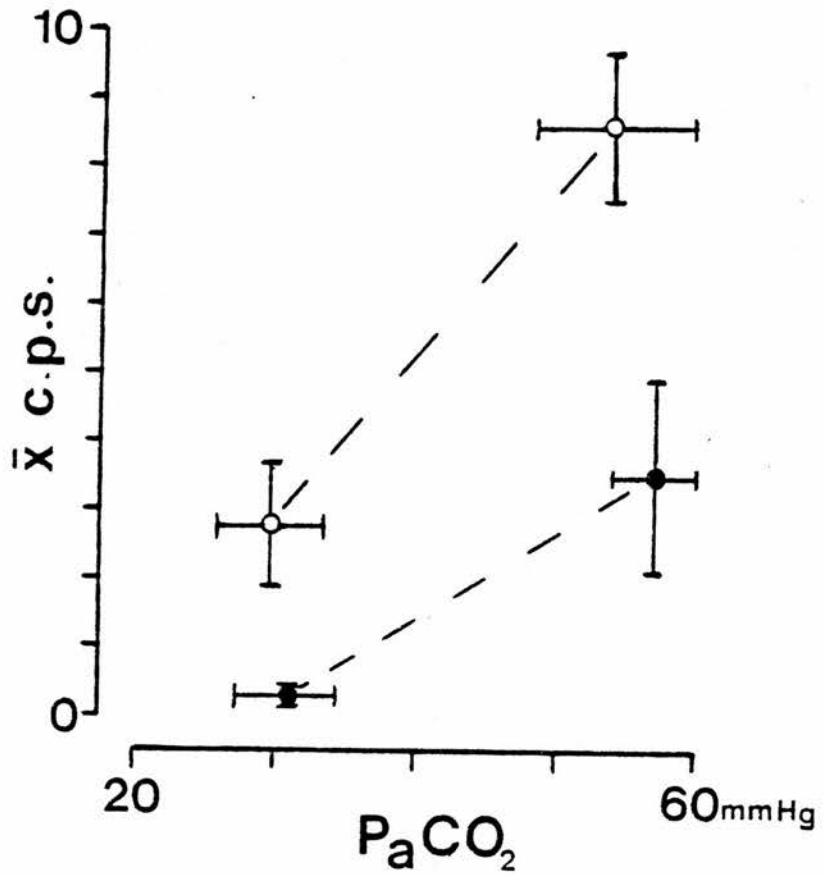


Figure 8.10. Chemoreceptor responses to hypercapnia. Results presented are pooled data from three separate experiments. Discharge (mean c.p.s. during steady state) has been plotted against $PaCO_2$. Results were obtained during infusion of Locke infusion (open symbols, ± s.e.m.) and during infusion of [Met]enk (2 μg min^{-1}, n=1, and 10 μg min^{-1}, n=2 - closed symbols, ± s.e.m.). [Met]enk infusion depressed rate of discharge during both normo- and hyper- capnia.

might be judged to have increased during infusion of [Met]enk since the increase in discharge caused by hypercapnia during infusion of the opioid was twelve-fold (1383 ± 864%), but only three-fold (244 ± 63%) during Locke infusion. $PaCO_2$ values during normocapnia and hypercapnia were unaltered by the infusion of [Met]enk.

The antagonist ICI 154129 had no appreciable effect upon the response to hypoxia in three experiments (e.g. Fig. 8.11), and none of the parameters used to assess the response to hypoxia was significantly different after the antagonist (table 8.4A).

The response to hypoxia was increased after ICI 174864 (0.1 or 0.2 mg kg^{-1}) in three out of four experiments (e.g. Fig. 8.12). There was a consistent tendency for 'plateau' level of discharge to increase, and there was an increase in the slope of the line representing the dynamic phase of the chemoreceptor response to hypoxia (table 8.4B). The low number of results and variability in magnitude of discharge (but not in overall pattern of altered response) meant that only control (pre-hypoxia) discharge was significantly altered after the antagonist.

Both antagonists affected the chemoreceptor response to three levels of hypercapnia at normal PaO_2. When plotted against $PaCO_2$, mean discharge frequency during steady-state was lower at all levels of $PaCO_2$ after ICI 154129 (10 mg kg^{-1}, n=2) or ICI 174864 (0.2 mg kg^{-1}, n=2)- see Figs 8.11 & 8.12.

8.3.4 Interactions of monoamines and [Met]enkephalin.

In this series of experiments monoamines were injected during infusion of [Met]enk, and the effects of each injection were analysed as previously described (see Sections 2 and 3). In general the

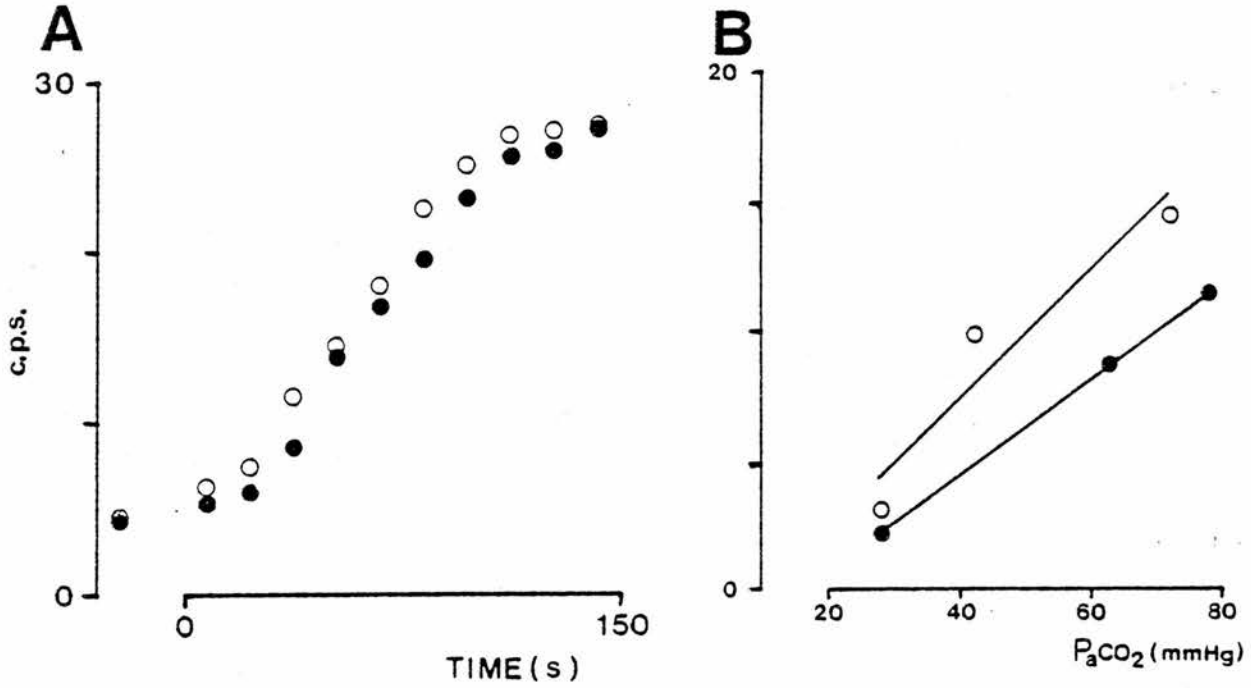


Figure 8.11. (A) Chemoreceptor responses to 10% O_2-hypoxia in a single experiment, before (open symbols) and after (closed symbols) administration of the antagonist ICI 154129, 10 mg kg^{-1}. Basal and plateau levels of discharge were not significantly altered, nor was the slope of the dynamic part of the response to hypoxia, shown here. Discharge (c.p.s.), averaged in successive 15 s intervals, has been plotted against time, 0 s indicating the onset of hypoxia; the mean pre-hypoxia control discharge is also indicated.

(B) The response of chemoreceptors, in the same experiment, to three levels of CO_2 at the same level of PaO_2. Mean discharge during steady-state at each level of CO_2 has been plotted against $PaCO_2$. Open symbols represent data obtained before, and closed symbols data obtained after injecting the antagonist ICI 154129, 10 mg kg^{-1}.

Lines were fitted to the data using the method of least squares.

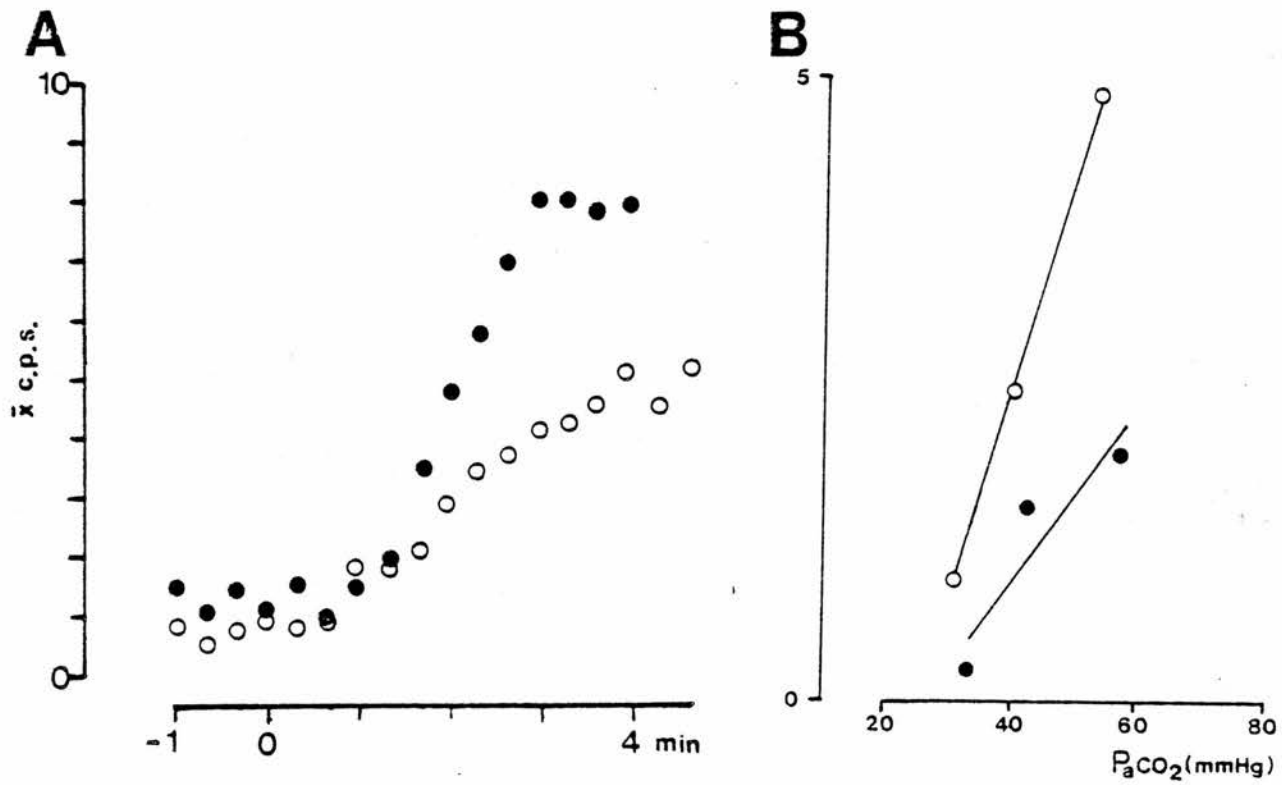


Figure 8.12. Chemoreceptor responses to 10% O_2-hypoxia in a single experiment, before (open symbols) and after (closed symbols) administration of the antagonist ICI 174864, 0.2 mg kg^{-1}. Basal and plateau levels of discharge were significantly increased, as was the slope of the dynamic part of the response to hypoxia. Discharge (c.p.s.), averaged in successive 15 s intervals, has been plotted against time, 0 s indicating the onset of hypoxia.

(B) The response of chemoreceptors, in the same experiment, to three levels of CO_2 at the same level of PaO_2. Mean discharge during steady-state at each level of CO_2 has been plotted against $PaCO_2$. Open symbols represent data obtained before, and closed symbols, data obtained after injecting the antagonist ICI 174864, 0.2 mg kg^{-1}.

Lines were fitted to the data using the method of least squares.

Table 8.4: Effects of opioid antagonists upon parameters defining the responsiveness of chemoreceptors to hypoxia; (A) measurements before and after the antagonist ICI 154129 (10 mg kg^{-1}) and (B) in a separate set of experiments, before and after ICI 174864 (0.2 mg kg^{-1})

A	Control	After ICI 154129	mean difference (± s.e.m.)	n
Control $\bar{x}$ c.p.s.	5.4	4.8	0.6 ± 1.8	3
Plateau $\bar{x}$ c.p.s.	27.7	20.7	7.0 ± 10.1	3
N_2 peak $\bar{x}$ c.p.s.	39.7	35.2	4.4 ± 12.7	3
Slope (% max s^{-1})	0.96	0.82	0.15 ± 0.30	3
Time to plateau (s)	103	102	1.0 ± 1.0	3
Control c.p.s. %	20	22	2.1 ± 7.0	3
Plateau c.p.s. %	100	93	7.0 ± 30.6	3
N_2 peak c.p.s. %	165	166	1.7 ± 35.6	3
PaO_2 (10% O_2; mm Hg)	33.7	34.3	0.67 ± 2.6	6
$PaCO_2$ (mm Hg)	32.0	28.6	3.4 ± 2.4	6
pHa	7.33	7.34	0.007 ± 0.018	6

B	Control	After ICI 174864	mean difference (± s.e.m.)	n
Control $\bar{x}$ c.p.s.	1.7	2.7	1.1 ± 0.3	3
Plateau $\bar{x}$ c.p.s.	8.9	12.4	3.4 ± 1.6	3
N_2 peak $\bar{x}$ c.p.s.	18.5	18.7	0.2 ± 4.1	3
Slope (% max s^{-1})	0.58	0.91	0.33 ± 0.3	3
Time to plateau (s)	166	186	19.7 ± 51.5	3
Control c.p.s. %	19.7	32.3	12.7 ± 1.8 *	3
Plateau c.p.s. %	100	163	63.3 ± 29.2	3
N_2 peak c.p.s. %	216	254	38.0 ± 58.0	3
PaO_2 (10% O_2; mm Hg)	43.8	41.8	2.0 ± 5.8	3
$PaCO_2$ (mm Hg)	27.8	29.7	1.8 ± 1.9	3
pHa	7.34	7.34	0.002 ± 0.023	3

* $P<0.05$ (Student's paired t-test.)

background discharge at the time of injection was decreased during infusion of the opioid, and raised during infusion of NAL, relative to control discharge during infusion of Locke solution.

Dose response data were obtained during Locke, [Met]enk or NAL infusion, and the integrated reponses plotted as sets of dose response curves; it was noted from the relative positions of these curves whether the integrated response was unaltered, increased or reduced during [Met]enk or NAL infusion, relative to the curve obtained during Locke infusion. The mean pre-injection control discharge of all tests included in each dose-response curve was calculated and the effects of drug infusions were compared with the control discharge during Locke infusion by expressing this mean value as a percentage change ($\bar{x}\Delta\%$) from the corresponding control mean. The standard error of any set of pre-injection control data was generally small, and the change in background discharge against which injections of monoamines was tested for statistical significance.

For each set of dose response curves the magnitude of the shift in response was assessed by expressing the response to each injection of a monoamine as a percentage change from the effect elicited by the same dose of drug during Locke infusion (average change - av$\Delta\%$). This gave a set of three to four values for each dose-response curve which were highly variable in every case; the average of these values, discarding any obvious anomalies, was taken as an index of the change in responsiveness of the system. The parameter $\bar{x}\Delta\%$ was used to predict the amount of change in the dose-relationship of the integrated response that could be accounted for by the change in background discharge caused by infusion of [Met]enk or NAL; when compared with this, av$\Delta\%$ gave an indication of whether or not the

observed changes in the magnitude of $\Delta\Sigma x$ were likely to result from attenuation or potentiation of responses by the opioid or the antagonist.

Examples of the changes in integrated responses to injections of NA, DA, ACh and 5-HT during infusions of [Met]enk or NAL are shown in Figs 8.13-8.16. The calulated values of $\bar{x}\Delta\%$ and $av\Delta\%$ for dose-response data to these monoamines are shown in tables 8.5-8.8. There was no clear difference in the changes in background discharge caused by infusion of [Met]enk at a concentration of either 2 or 10 $\mu g\ min^{-1}$. Where $\bar{x}\Delta\%$ and $av\Delta\%$ were comparable (with a difference of less than 10-15%) it was considered that the change in magnitude of the response to the injected drug was due to alterations in background discharge caused by the infused drug. If these two measurements differed it was inferred that the change reflected some degree of interaction. Results are summarised in table 8.9. Where DA- or 5-HT- evoked chemodepression was altered during infusion of [Met]enk, it was generally potentiated. No clear pattern of change in the depressant response to NA emerged. Some 50-75% of all depressant responses, whether evoked by NA, DA or 5-HT were unaltered during infusion of [Met]enk. Excitatory responses to ACh were almost equally unaltered or potentiated. Secondary excitatory responses to NA were potentiated in over half of the tests.

Delayed excitation following 5-HT-evoked chemodepression was unaltered in one experiment and potentiated in the other. Initial transient excitation, where present, was still seen to occur during [Met]enk infusion, but was not quantified as the effect is normally variable, and was clearly small during infusion of the peptide.

During infusion of NAL responses to all monoamines were altered; chemodepression evoked by NA was potentiated in all cases (see table

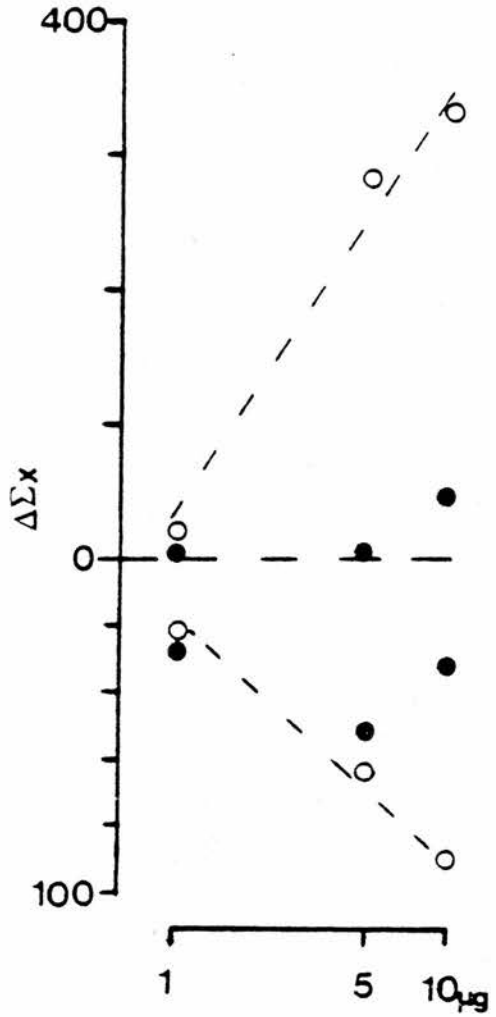


Figure 8.13. Dose-response data (integrated responses - $\Delta\Sigma x$ - plotted against dose) for the initial chemodepressant and secondary excitatory effects of NA injection during infusion of Locke solution (0.1 ml min^{-1}, open symbols) and [Met]enk (10 μg min^{-1}, closed symbols). The opioid caused a mean depression of background discharge ($\bar{x}\Delta\%$) of -62%. Chemodepression was reduced by a factor of approximately -42% (av$\Delta\%$), and chemoexcitation was reduced by about 87%, thus the reduction in both depression and excitation could not be explained solely as being due to the 62% reduction in basal discharge, against which the injections were made.

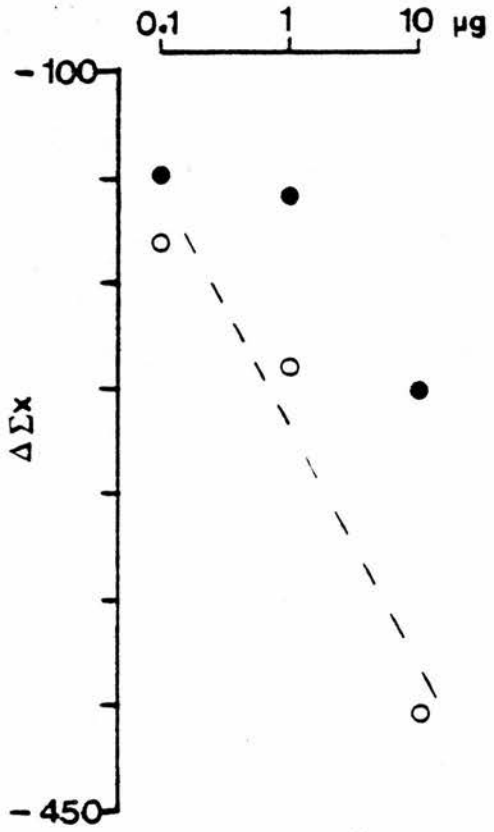


Figure 8.14. Dose-response data (integrated responses - $\Delta\Sigma x$ - plotted against dose) for the chemodepressant effects of DA injection during infusion of Locke solution (0.1 ml min^{-1}, open symbols) and [Met]enk (10 µg min^{-1}, closed symbols). The opioid caused a mean depression of background discharge ($\bar{x}\Delta\%$) of -42%. Chemodepression was reduced by a factor of approximately -30% (av$\Delta\%$), and the reduction in depression is possibly accounted for by the 42% reduction in basal discharge by opioid infusion, against which the injections were made.

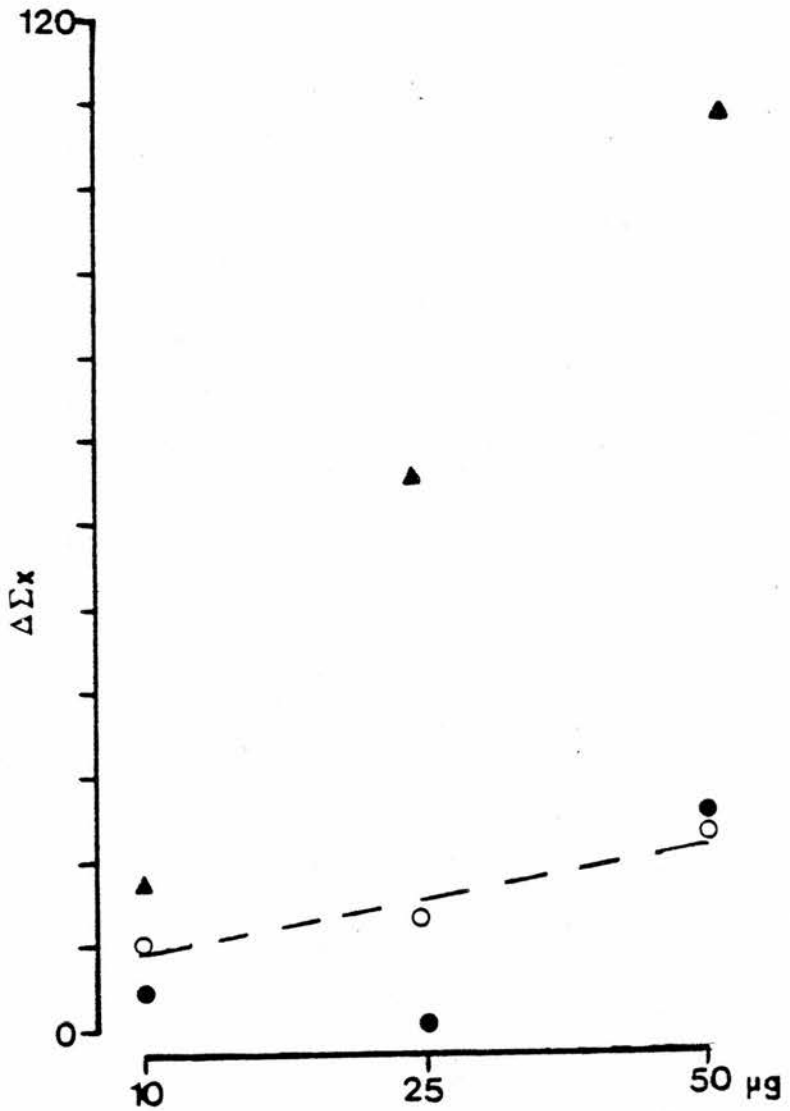


Figure 8.15. Dose-response data (integrated responses - $\Delta\Sigma x$ - plotted against dose) for the excitatory effects of ACh injection during infusion of Locke solution (0.1 ml min^{-1}; O), [Met]enk (10 µg min^{-1}; ●) and NAL (600 µg min^{-1}; ▲). The opioid caused a mean depression of background discharge ($\bar{x}\Delta\%$) of -22%. Chemoexcitation was reduced by a factor of approximately -19% (av$\Delta\%$) during opioid-infusion, and the reduction in excitation could be explained solely as being due to the 22% reduction in basal discharge, against which the injections were made. During NAL infusion background discharge was increased by some 130%, relative to Locke infusion control discharge, but the ACh-evoked excitation was increased by some 315% when compared to Locke infusion controls, suggesting that the effect was potentiated during infusion of the opioid antagonist.

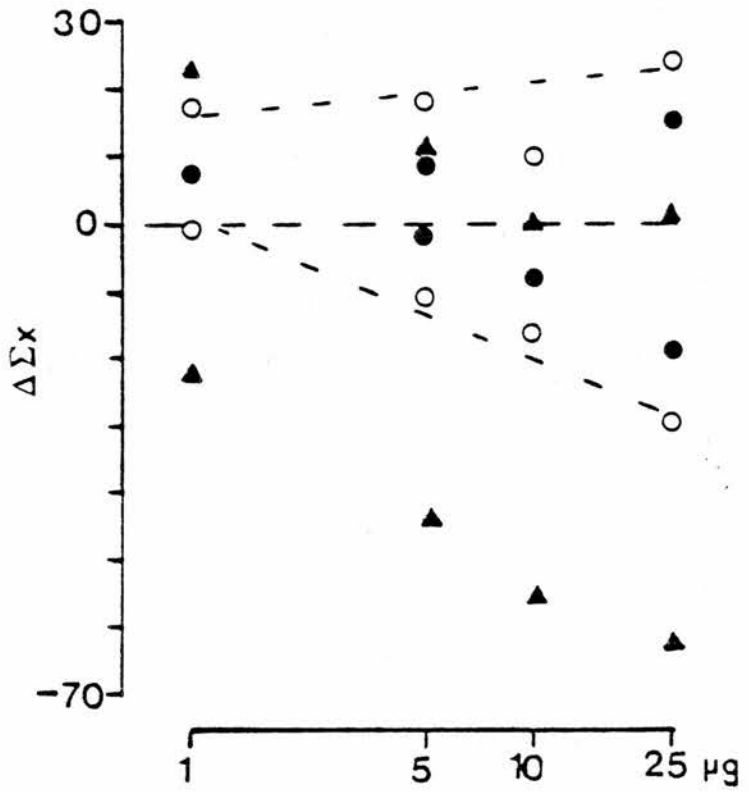


Figure 8.16. Dose-response data (integrated responses - $\Delta\Sigma x$ - plotted against dose) for the chemodepressant and delayed excitatory effects of 5-HT injection during infusion of Locke solution (0.1 ml min^{-1}; O), [Met]enk (10 µg min^{-1}; ●) and NAL (600 µg min^{-1}; ▲). The opioid caused a mean depression of background discharge ($\bar{x}\Delta\%$) of -53%. Chemoexcitation was reduced by a factor of approximately -47% (av$\Delta\%$) and chemodepression by a factor of some -56% during opioid-infusion, and these reductions might be explained solely as being due to the ~50% reduction in basal discharge, against which the injections were made. During NAL infusion background discharge was increased by some 117%, relative to Locke infusion control discharge; chemodepression evoked by 5-HT was increased approximately 675%, and chemoexcitation was reduced. The depressant effect of 5-HT during NAL infusion was potentiated during infusion of the opioid antagonist since the percentage change, whilst lacking precision, is none-the-less far greater than can be accounted for solely by the shift in background discharge.

Table 8.5: Assessment of the effects of simultaneous infusion of [Met]enk or naloxone upon the response of chemoreceptors to i.c. injection of noradrenaline in eight experiments.

[Met]enk 2 µg min^{-1}					[Met]enk 10 µg min^{-1}					NAL 600 µg min^{-1}				
$\bar{x}\Delta\%$	av$\Delta\%$		Net effect		$\bar{x}\Delta\%$	av$\Delta\%$		Net effect		$\bar{x}\Delta\%$	av$\Delta\%$		Net effect	
	Dep	Exc	Dep	Exc		Dep	Exc	Dep	Exc		Dep	Exc	Dep	Exc
-57*	-78	-29	↓	↑						105*	555	-49	↑	↓
-76*		5		↑										
-24*	-27	84	=	↑						129*	231	37	↑	↓
					-53*	-40	-62	=	=					
					-62*	-42	-87	↑	↓					
					-54*	-62	-20	=	↑					
										135*	430	283	↑	↑
										143*	348	-23	↑	↓

$\bar{x}\Delta\%$: pre-injection control ([Met]enk infusion) expressed as percentage change from pre-injection control (Locke infusion). av$\Delta\%$: average change from Locke infusion values. Exc: chemoexcitation. Dep: chemodepression. = no change; ↑ increased; ↓ decreased. * $P < 0.05$ (Student's t-test).

Table 8.6: Assessment of the effects of simultaneous infusion of [Met]enk or naloxone upon the response of chemoreceptors to i.c. injection of dopamine in ten experiments.

[Met]enk 2 μg min^{-1}			[Met]enk 10 μg min^{-1}			NAL 600 μg min^{-1}		
$\bar{x}\Delta\%$	av$\Delta\%$ Dep	Net effect Dep	$\bar{x}\Delta\%$	av$\Delta\%$ Dep	Net effect Dep	$\bar{x}\Delta\%$	av$\Delta\%$ Dep	Net effect Dep
-19	38	↑	-48	-19	↑	267	372	↑
-85*	-75	=	-92*	-85	=			
-24	32	↑						
-81*	-83	=						
-34	-18	↑						
-16	-73	↓						
			-42*	-30	=			
			-67*	-52	=	305*	241	↓
			-38*	-32	=	-7	21	↑
						143	348	↓

$\bar{x}\Delta\%$: pre-injection control ([Met]enk infusion) expressed as percentage change from pre-injection control (Locke infusion). av$\Delta\%$: average change from Locke infusion values. Dep: chemodepression. = no change; ↑ increased; ↓ decreased.
* $P < 0.05$ (Student's t-test).

Table 8.7: Assessment of the effects of simultaneous infusion of [Met]enk or naloxone upon the response of chemoreceptors to i.c. injection of acetylcholine in eight experiments.

[Met]enk 2 µg min^{-1}			[Met]enk 10 µg min^{-1}			NAL 600 µg min^{-1}		
$\bar{x}\Delta\%$	av$\Delta\%$ Exc	Net effect Exc	$\bar{x}\Delta\%$	av$\Delta\%$ Exc	Net effect Exc	$\bar{x}\Delta\%$	av$\Delta\%$ Exc	Net effect Exc
-54*	10	↑						
-23	-44	↓	-24	17	↑			
-44	-36	=						
-77*	47	↑				77*	-25	↓
			-62*	-62	=			
			-22	-19	=	130*	315	↑
			-41*	-35	=			
			-83*	-52	↑	19	-67	↓

$\bar{x}\Delta\%$: pre-injection control ([Met]enk infusion) expressed as percentage change from pre-injection control (Locke infusion). av$\Delta\%$: average change from Locke infusion values. Exc: chemoexcitation. = no change; ↑ increased; ↓ decreased.
* $P < 0.05$ (Student's t-test).

Table 8.8: Assessment of the effects of simultaneous infusion of [Met]enk or naloxone upon the response of chemoreceptors to i.c. injection of 5-hydroxytryptamine in five experiments.

[Met]enk 2 μg min^{-1}					[Met]enk 10 μg min^{-1}					NAL 600 μg min^{-1}				
$\bar{x}\Delta\%$	av$\Delta\%$		Net effect		$\bar{x}\Delta\%$	av$\Delta\%$		Net effect		$\bar{x}\Delta\%$	av$\Delta\%$		Net effect	
	Dep	Exc	Dep	Exc		Dep	Exc	Dep	Exc		Dep	Exc	Dep	Exc
					-9	8	0	=	0					
					-54	-51	0	=	0	-21	-63		↓	
-53*	-56	-47	=	=						117*	675	-48	↑	↓
										490*	137	-8	↓	↓
-31	-4	-13	↑	↑										

$\bar{x}\Delta\%$: pre-injection control ([Met]enk infusion) expressed as percentage change from pre-injection control (Locke infusion). av$\Delta\%$: average change from Locke infusion values. Dep: chemodepression; Exc: chemoexcitation. = no change; ↑ increased; ↓ decreased. * $P < 0.05$ (Student's t-test).

Table 8.9: Summary of the changes in effects upon chemoreceptor discharge of injected monoamines caused by infusion of (A) [Met]enkephalin (2-10 μg min^{-1}) or (B) naloxone (600 μg min^{-1}).

(A) Effects of [Met]enk.

	no change	potentiation	attenuation	n
NA				
Depression	60%	20%	20%	5
Excitation	16.5%	67%	16.5%	6
DA				
Depression	55%	36%	9%	11
ACh				
Excitation	44.5%	44.5%	11%	9
5-HT				
Depression	75%	25%	0%	4
Delayed excitation	50%	50%	0%	2

(B) Effects of NAL.

	no change	potentiation	attenuation	n
NA				
Depression	0%	100%	0%	4
Excitation	0%	25%	75%	4
DA				
Depression	0%	67%	33%	3
ACh				
Excitation	0%	33%	67%	3
5-HT				
Depression	0%	33%	67%	3
Delayed excitation	0%	0%	100%	2

8.3.5 Summary of results presented in section 8.

1. Opioid peptides and related drugs caused chemodepression. The rank order of potency as chemodepressants was [Met]enk ≥ [Leu]enk = DADL > DAGO >> DYN >> EKZ >>> MCPT = 0.

2. Chemodepression was reduced by the selective 'δ'-opioid antagonists ICI 154129 and ICI 174864 in a dose related manner.

3. Infusion of [Met]enk suppressed the increase in discharge elicited by ventilation with hypoxic or hypercapnic gas mixtures. The response to hypoxia was unaltered after ICI 154129, but potentiated after ICI 174864. The response to hypercapnia was reduced after administration of either of these two selective antagonists.

4. During infusions of [Met]enk chemodepression evoked by NA was equally potentiated or attenuated, but was unaltered in 60% of experiments. Delayed excitatory effects of NA were potentiated in 67% of recordings. DA-evoked chemodepression was potentiated in 36% of recordings, but unaltered in 55%, whilst chemodepression in response to 5-HT was largely unaltered. ACh-evoked excitation was potentiated or unaltered with equal frequency during infusion of the peptide.

5. During infusions of NAL chemodepression evoked by NA was potentiated in all recordings and DA-evoked chemodepression was potentiated in 67% of recordings. Delayed excitatory effects of NA were attenuated in 75% of recordings. ACh-evoked excitation was attenuated in 67% of recordings, during infusion of the peptide. Chemodepression in response to 5-HT was usually attenuated, as was delayed excitation.

8.9) and the effect of DA was potentiated in two out of three experiments. Depression caused by 5-HT was, however, more commonly attenuated. Excitatory effects of NA were attenuated during NAL infusion in three out of four experiments, since the change in $\Delta\Sigma x$ was less than anticipated from the increase in background discharge, during infusion of the antagonist. ACh-evoked chemoexcitation was attenuated in two out of three experiments, but the delayed excitatory response to 5-HT was attenuated in both experiments during infusion of NAL.

8.3.5 Summary.

8.4 Discussion.

The pattern of response of cat carotid chemoreceptors to exogenous [Met]enk accorded well with that reported previously by McQueen and Ribeiro (1980, 1981b). Chemodepression evoked by this peptide is similar to the response evoked by the catecholamine DA, although at the doses used the effect of the opioid was rather more prolonged, discharge remaining depressed for many seconds, and up to one or two minutes with the highest doses used in these experiments. [Met]enk is a prototype δ-opioid agonist, and its effects are less complex than those of β-END or morphine (McQueen and Ribeiro, 1980, 1981b) which are relatively weak modulators of chemoreceptor activity, even though they appear capable of activating several different sub-types of opioid receptor (cf. Section 1). The antagonist NAL is more potent at μ-receptors and the only partial antagonism of [Met]enk-evoked chemodepression by NAL, even at high doses of the antagonist (which more effectively block the effects of β-END), suggests a selective action of [Met]enk at a receptor other than the μ-sub-type.

The evidence provided by the experiments reported here appears to

confirm that the chemodepressant effects of enkephalins are mediated mainly through actions at δ-receptors. Agonists which are considered to be relatively more selective for μ- and κ- receptors (but not devoid of actions at δ-receptors - cf. Paterson et al, 1983) were less potent (κ-agonists markedly so) than either the enkephalins or the 'selective' δ-agonist DADL. Furthermore, there is the evidence provided by the actions of the two selective δ-antagonists, ICI 154129 and ICI 174864, both of which effectively blocked the effects upon chemoreceptor discharge of enkephalins. ICI 154129 is some thirty times more selective for δ-receptors than for μ-receptors (Shaw et al, 1982) and ICI 174864 is devoid of actions at receptors other than δ-receptors when used at concentrations of less than 5 μM in vitro. In the experiments reported here the maximum amount of this antagonist administered in any one animal ($0.1+0.2$ mg kg^{-1}, or approximately 0.5 μM) was very much less than the amount likely to show non-selective actions at other opioid receptors, but it did virtually completely block the chemodepressant effects of δ-agonists. It is reasonable to conclude that the predominant effects of opioids upon chemoafferent activity are mediated through actions at δ-opioid receptors.

[Met]enk was slightly more potent (although the difference was not significant) than [Leu]enk in these experiments, although the latter is generally considered to be the more potent agonist in the mouse vas deferens (e.g. Waterfield et al, 1979). Whether this results from the differing numbers of experiments carried out with the two pentapeptides (13 experiments with [Met]- and 5 experiments with [Leu]- enk) or reflects some property related to the greater abundance of the [Met] form in the carotid body (Wharton et al, 1980) cannot be determined. It was observed that the sensitivity to [Met]- or [Leu]-

enk often depended upon the order in which the two drugs were administered, so that chemoreceptors were apparently more sensitive to the [Leu]- form if this agonist was studied first, somewhat less sensitive if [Met]enk was the first opioid injected. Further experiments would be required to elucidate whether there is some partial desensitisation after the first application of an opioid. Possibly this could involve some form of irreversible binding at some of the δ-receptors, or an allosteric change in part of the receptor population.

Enkephalins have been proposed as inhibitory transmitters since they show hyperpolarising actions when applied to some peripheral and central neurones (e.g. North et al, 1979; Pepper & Henderson, 1980). This hyperpolarising action has not been observed in all preparations (e.g. Zieglgänsberger & Tulloch, 1979) and some allowance must also be made for a possible modulatory role of enkephalins whereby the actions of other transmitters are altered. This could represent a change in the release of transmitters (e.g. monoamines - cf. Henderson, 1983) or changes in the effects of other transmitters at their own post-synaptic sites of action.

Opioids cause dose-related, NAL-reversible hyperpolarisations when applied to locus coeruleus neurones (Pepper & Henderson, 1980); the effect appears to represent a direct action upon the neurone since it is unaltered by abolition of synaptic activity by perfusion with a Ca^{2+}-free medium. Such hyperpolarisation is associated with an increased Ca^{2+}-sensitive K^+ conductance (Williams et al, 1982). Opioids have been reported to reduce ATP-dependent binding of Ca^{2+} to synaptosomal membranes (Guerrero-Munoz et al, 1979), and binding of opioids to opioid receptors could result in either displacement of

Ca^{2+} from binding sites on the inner plasma membrane, or in decreased sequestration of Ca^{2+} entering a neurone during activation, thus causing an increased intracellular $[Ca^{2+}]$ activating an increase in K^+ conductance (see Henderson, 1983). Inhibition of K^+-evoked NA release from slices of occipital cortex and hypothalamus by [Met]enk appears to involve a reduction in the transmembrane inward current carried by Ca^{2+} ions (Göthert et al, 1979). The opioid receptors mediating adenylate cyclase inhibition in cultured tumour cells of neural origin are of the δ-subtype (Chang & Cuatrecasas, 1979; McLawhon et al, 1981; Gilbert et al, 1982), and opioid inhibition of adenylate cyclase in striatal tissue is also possibly mediated through δ-receptors (Cooper et al, 1982). This is of interest since the DA D_2-receptor may also be negatively-linked to adenylate cyclase (Creese et al 1982), and could represent a common mechanism for depression of chemoreceptor activity (see later). It is perhaps an anomaly of the carotid body that α-receptors that supposedly also inhibit adenylate cyclase activity when activated do not appear to mediate chemodepression (see Sections 3, 4, & 6).

Delayed chemoexcitatory responses to enkephalins were not very evident in the experiments reported here, regardless of the agonist used; after δ-receptor blockade there was no obvious or consistent replacement of chemodepression with an excitatory effect, and it could be that the chemoexcitatory responses to exogenous opioids seen occasionally in vivo (e.g. McQueen & Ribeiro, 1980, 1981b) result from indirect changes effected outwith the carotid body, thus explaining their inconstancy. There is some important evidence from a recent in vitro study (Monti-Bloch & Eyzaguirre, 1985) which very much contrasts with this speculation. These authors reported that

superfusion of the carotid body with [Met]enk-containing solutions caused chemodepression followed by 'rebound' excitation; injection of bolus doses of [Met]enk in this preparation caused chemoexcitation at low doses and depression at high doses. The possibility exists that both 'inhibitory' and 'excitatory' receptors are present in the cat carotid body, as in the rat vas deferens, where morphine can cause both inhibitory and excitatory effects. The situation then becomes analogous to the comparable effects of DA in the carotid body, where depressant effects predominate in the in vivo preparation (and excitatory effects may sometimes by observed after DA-receptor blockade - see Section 1), but excitatory effects are far more common in the excised in vitro preparation (e.g. Zapata 1975).

One obvious inferenceis that some trophic neural activity (ganglioglomerular or efferent fibres supplied by the sinus nerve) is required to maintain the integrity of the systems that mediate the depressant effects of opioids or DA, which possibly exert a tonic 'braking' effect upon chemoreceptors. As after domperidone, background discharge increased during infusion of NAL; McQueen and Ribeiro (1980) reported that injection of NAL had only slight effects upon spontaneous discharge. In the present experiments infusion of NAL virtually always caused a significant increase in the background rate of chemoreceptor discharge. It is not possible to determine whether this results from 'disinhibition' of the chemoreceptors, or from the unmasking of excitatory responses to opioids at NAL-insensitive receptors. Presumably the sensitivity of both opioid- and DA- excitatory receptors is much lower than the sensitivity of receptors mediating chemodepression. Where two 'opposing' receptor mechanisms coexist low doses of agonist may activate both systems

simultaneously with no net change in ongoing activity; increasing doses of agonist may be expected to elicit greater responses through preferential activation of the more sensitive site - if the site mediating the greater activity is blocked then actions mediated through the 'complimentary' site become more evident. It seems unlikely that endogenous receptor 'antagonists' could play such a modulatory role, and the effect of interactions between differing neurotransmitter systems or the effects of (neuro)hormones are likely to be crucial. It is debateable whether two populations of receptors would exist in the same effector complex (i.e. at the same post-synaptic site) or whether two populations of effector mechanisms would co-exist mediating opposing effects in separate systems which would converge upon a common integrating element.

Palmer and Hoffer (1980) found that enkephalin-induced neuronal depression in rat frontal cortex was dependent upon the integrity of catecholamine afferents supplying the region, and concluded that the interaction here took the form of an augmentation of the post-synaptic effects of ENK by catecholamines. In the carotid body in vitro the loss of effective catecholaminergic control of the effects of opioids could explain the greatly differing results obtained, as compared with in vivo experiments, but it should be remembered that in most cases in situ the sympathetic nerves supplying the carotid body are cut, and the sinus nerve itself is cut, prior to recording; it thus remains possible that circulating substances may also play some important trophic role, or that they are necessary for the maintainance of activity at the terminals of efferent nerves, which can thus be prolonged (after axonal transection) for a longer period in the intact animal than when the carotid body is excised.

The scheme adopted for investigating possible interactions between opioids (and other peptides) and monoamines was based upon the assumption that peptides might alter the responsiveness to 'sporadically' or 'discretely' released classical transmitters, for instance by resetting resting membrane potential, increasing or reducing the likelihood that a given ammount of transmitter will produce its normal effects.

When examined subjectively, dose-response curves for the effects of monoamines drawn from data obtained during [Met]enk infusion showed either no change in the position of the data points, relative to those obtained during Locke infusion, or a reduction in the magnitude of the integrated reponse ($\Delta\Sigma x$) at any given dose. This was true whether considering depressant or excitatory effects. The pattern of response to all monoamines studied appeared not to vary, whether injections were made during infusion of Locke solution, [Met]enk, or NAL; the time course of the various phases of the responses was also not seen to be altered. Much care is needed in the interpretation of such data; the integrated response represents an absolute number of action potentials occuring additionally, or failing to occur, in a given period of time when a calculated number of action potentials is predicted, supposing no alteration in mean discharge frequency. If the numerical value of $\Delta\Sigma x$ remains constant (with no marked changes in the time course of the response) yet background discharge is reduced (as during [Met]enk infusion) then the responsiveness of the system can be said to have increased. Where control discharge is reduced, the reduction in the magnitude of $\Delta\Sigma x$ may represent no net change in responsiveness, unless the degree of reduction in $\Delta\Sigma x$ exceeds the amount of decrease in control discharge. It was considered necessary, then,

to attempt to quantify the changes in responses to monoamines during opioid or opioid antagonist infusions, to determine whether the altered effects of monoamine injections reflected changes in basal discharge frequency, or could represent some change in the sensitivity of the chemoreceptors to these substances.

The dose-response curves compared were all linear relationships (deviation from linearity in the case of NA excitation - cf. Section 3 - was avoided by restricting the range of doses used), but not parallel. In very general terms dose-response curves were shifted by a constant factor, suggested by the fact that the percentage change from the control curve was usually comparable at at least two doses; anomalies frequently arose because of deviations from a best-fit line drawn through the data points, which could occur in either or both of the curves in each pair that were compared. Also, since only three to four data points were obtained for each dose-response curve, even small variations (i.e. poor correlation) led to errors in calculating a mean factor quantifying the change in position of the curve during [Met]enk or NAL infusion. The analysis carried out in these experiments is limited in its precision, and may represent an underestimation of the number of experiments where [Met]enk altered the response of the chemoreceptors to the injected drugs.

Subjectively it appeared that infusion of [Met]enk caused a reduction in the integrated responses to monoamines, and NAL infusion caused an increase. Where the mean change in background frequency (mean pre-injection control discharge for all points contributing to a dose-response curve during drug infusion expressed as a percentage change from the mean of the same points during Locke infusion - $\bar{x}\Delta\%$) appeared to be close to the average percentage change of the dose-res-

ponse data from control values, it was concluded that the change in position of the dose-response curve during drug infusion could be accounted for by the alterations in background rate of discharge. Just over half of the depressant responses to DA and NA (which may be mediated through activation of the same receptors) were considered, on these grounds, to be unaltered during infusion of [Met]enk, although chemoreceptor discharge was reduced by the opioid. There was greater evidence for a potentiation of the depressant effect of DA than for an attenuation, during opioid infusion. Given that the majority of depressor responses to these catecholamines were unaffected, the apparent potentiation of some responses may represent an additive effect of opioid- and catecholamine- evoked chemodepressions. The failure of two depressant effects to summate (in the case of experiments where there was no obvious change in the responsiveness to catecholamines) might also be interpreted as an interaction, since it would appear that one form of depression is not contributing to the anticipated net effect.

Chemodepression evoked by 5-HT was reduced during [Met]enk infusion, but in 75% of experiments it seemed that the shift in the dose-response curve could be accounted for by the similar reduction in background level of discharge at the time of injection. In one experiment only there was evidence of a potentiation of the chemodepressant effect.

Delayed or secondary excitation followed the depression of discharge evoked by NA and 5-HT. In the case of NA there was clear evidence for a potentiation of the effect in more than half of the experiments performed during infusion of [Met]enk. Only two experiments with 5-HT gave consistent results showing a dose-related

secondary chemoexcitatory effect and it should be remembered that this effect is more commonly observed only after blockade of depressant effects (Section 7). Both types of excitatory responses examined here have been more fully discussed in earlier sections of this thesis, where it was concluded that they are largely dependent upon the development of cardiovascular changes, particularly in the case of NA. Since [Met]enk infusion produced a fall in systemic blood pressure, which commenced at least a minute before injections of the monoamines, it is possible that reflex mechanisms could already have begun to alter cardiovascular activity by the time of injection, hence the potentiation of the NA-evoked delayed excitation. The main factor against such an interpretation is the fact that reflex activity is generally not present during pentobarbitone-anaesthesia. The more direct excitation caused by ACh was attenuated in one experiment only, during infusion of [Met]enk. In all other cases it was either unaltered or potentiated with equal frequency, and the events that underly this effect cannot be certainly identified.

If NAL infusion can be considered only to have blocked the effects of endogenously released opioids (cf. Sawynok et al, 1979), as suggested by the increase in background discharge, then the effects of concomitant NAL infusion upon monoamine responses may prove to be of some interest. In all cases the dose-response curve for monoamine effects was shifted to a degree markedly different from that predicted by the shift in background rate of discharge. Most striking was the potentiation of catecholamine-evoked chemodepression, which contrasted with the more usual reduction of 5-HT evoked chemodepression during NAL infusion. A possible interpretation of this finding is that (endogenous) opioids are normally responsible for limiting the depressant

effects of catecholamines. This raises the possibility that exogenous catecholamines depress chemoafferent activity through release of another (as yet unidentified) transmitter or, perhaps more likely, that there is an interaction at the level of the intracellular second messenger.

The majority of excitatory responses, whether evoked by NA, 5-HT or ACh, were attenuated during NAL infusion. This finding is again difficult to interpret, although it seems reasonable to suggest that if depressant effects are modulated in one direction then excitatory effects are likely to be affected reciprocally, since the two types of effect must surely represent complimentary biochemical mechanisms. Less clear is the correlation between reduction in the 'direct' effects of ACh and the reduction in the 'delayed' excitatory effects of NA or 5-HT. It is not possible from these experiments to assess any contribution from actions at excitatory NAL-resistant receptors stimulated by endogenously-released opioids. To reconcile such an effect with the results obtained is difficult, except by arguing that 'excitation'-mediating opioid receptors facilitate the release of transmitters, which under basal conditions are tonically excitatory; it would be necessary to show that when DA or NA are applied, they evoke release of inhibitory transmitters, this effect also being enhanced when 'excitatory'-opioid receptors are active. So far it has not appeared necessary to invoke release of second transmitters to explain the very rapid effects of catecholamines upon chemoreceptors, and it seems more reasonable, partly because of the rapidity of the effects of the injected monoamines, to suggest that interactions with opioid peptides are more likely to occur at the level of second messengers.

Adenylate cyclase is activated by guanosine triphosphate (GTP; Rodbell et al, 1971) and by non-hydrolysable analogues of GTP (Londos et al, 1977). Koski and Klee (1981) reported that opioid petides stimulate GTP hydrolysis, leading to inhibition of adenylate cyclase (the effect being reversed by NAL). Some substances, such as prostaglandin E_2, secretin, and fluoride can stimulate adenylate cyclase activity without affecting GTP hydrolysis (see Koski & Klee, 1981); others, notably β-agonists, stimulate both adenylate cyclase and the hydrolysis of GTP (Cassel & Selinger, 1976; Fain & García-Sáinz, 1980). Receptor-mediated inhibition of adenylate cyclase by α-adrenoceptor agonists, muscarinic agonists, and opioids appears to require the presence of Na^+ ions and GTP and it would most useful to confirm whether D_2-receptors are in fact negatively-linked to adenylate cyclase, as suggested by Creese et al (1982). The depressant effects of opioids and catecholamines might indeed share a common final pathway, with the addition that opioid actions could also reduce the capacity of catecholamines to inhibit adenylate cyclase. Sabol and Nirenberg (1979), studying mouse neuroblastoma-rat glioma hybrid cells, showed that the inhibition of adenylate cyclase caused by activation of α-receptors, muscarinic receptors (which mediate chemodepression in the rabbit - Docherty & McQueen, 1979) and opiate receptors was not additive, and they concluded that the three species of receptor can be functionally coupled to the same adenylate cyclase molecules or molecules regulating enzyme activity. GTP, which appears to be required for effecting responses to all three types of agent, was proposed as a coupling factor between receptors and the enzyme, and this coupling may result in activation or inhibition depending upon the receptor species.

If opioids are able to regulate such coupling by reducing GTP

levels then this could form a means of controlling D_2-receptors, if these are negatively-linked with adenylate cyclase and have a requirement for GTP to enable coupling, hence the enhanced chemodepression during NAL infusion, when GTP hydrolysis by opioids is 'inoperative'.

It would appear that further investigations of the possible interactions between monoamines and opioid peptides would prove worthwhile, but more refined quantification is required before a firm interpretation of the type of results obtained in this study can be attempted. The fact that the receptor modulating opioid effects upon the chemoreceptors has been shown probably to be of the δ-sub-type should facilitate future research, as more improved drugs become available for use in studies such as this.

SECTION 9

STUDIES ON THE EFFECTS OF SUBSTANCE P AND VASOACTIVE INTESTINAL POLYPEPTIDE IN THE CAT

SECTION 9.

STUDIES ON THE EFFECTS OF SUBSTANCE P AND VASOACTIVE INTESTINAL POLYPEPTIDE.

9.1 Studies on the effects of Substance P.

9.1.1 Introduction.

Evidence that Substance P (SP) modulates neural activity, is located in nerve fibres of the carotid body and can alter chemoreceptor activity, has been cited in Section 1. The effects of SP upon chemosensory activity are, however, equivocal (McQueen, 1980b), and warrant further investigation. From competitive binding studies, Iversen et al (1982) have proposed the existence of two distinct receptor sub-types - SP-P, which is equally sensitive to all the tachykinins, and SP-E, which is considerably more sensitive to eledoisin (ELD) and kassinin (KAS). The methyl ester of SP (SP-O-Me) and other alkyl esters of the undecapeptide appear to be relatively selective for the SP-P receptor (Iversen et al, 1982). Few potent and selective antagonists at SP-receptors have been identified, thus hindering the classification of SP-receptors, and the clarification of its possible role as a neurotransmitter.

The aim of this part of the study was to attempt a classification of the SP-receptors that might be involved in the response of chemoreceptors to the peptide, with the possibility that one of the other tachykinins might prove a more useful tool in further studying the effects of this type of peptide upon chemosensory activity. The tachykinins used were SP, SP-O-ME, ELD, KAS, physalaemin (PHY), and

Substance K (SK; isolated by Maggio et al - 1983 - from spinal cord, and proposed as a possible endogenous SP-E ligand; also identified as neurokinin α - Kimura et al, 1983).

9.1.2 Results.

9.1.2.1 Effects of injections of Substance P and other tachykinins.

Experiments were carried out in eight cats. SP (n=8), SK (n=3), PHY (n=3), KAS (n=3), SP-O-Me (n=1) and ELD (n=1) were injected over the dose-range 0.01 or 0.1 - 100 μg i.c. All caused a complex pattern of response. An initial depression of discharge lasting some 10 s was followed by a longer-lasting phase of increased chemoreceptor activity. Neither magnitude nor duration of either part of the response showed any marked dose-dependency, although the interval between injections was prolonged so as to avoid the effects of tachyphylaxis. The percentage-change from pre-injection control discharge levels was not dependent upon dose, and rarely exceeded 50% whether considering depressant or excitatory effects. None of the drugs tested showed any evidence of being more suitable than SP for use in subsequent experiments. Tachykinins were associated with a hypotensive effect, but this also was variable in its occurance and in its intensity.

9.1.2.2 Effects of Substance P infusions, and interactions with monoamines, hypoxia, and hypercapnia.

Two minute infusions of SP (10 μg min^{-1} i.c.; n=49) were made in four experments. Short infusions were performed so as to minimise the possibility of desensitising the preparation. Mean disharge frequency was calculated in successive 15 s intervals; within 15 s of onset of

SP infusion there was a small but significant decrease ($P<0.05$; Student's t-test) in the mean discharge frequency, when compared with the mean pre-infusion control discharge (Fig. 9.1). Infusion of Locke solution (0.1 ml min^{-1} i.c.; n=63) was not associated with any marked reduction in discharge frequency. The pattern of discharge during any infusion was variable, and it was found that in the case of Locke infusion approximately half of all the measurements of discharge frequency represented a slight increase, and half a slight decrease; for results obtained with SP infusion some 60% of results represented decreases from control, and 40% represented increases.

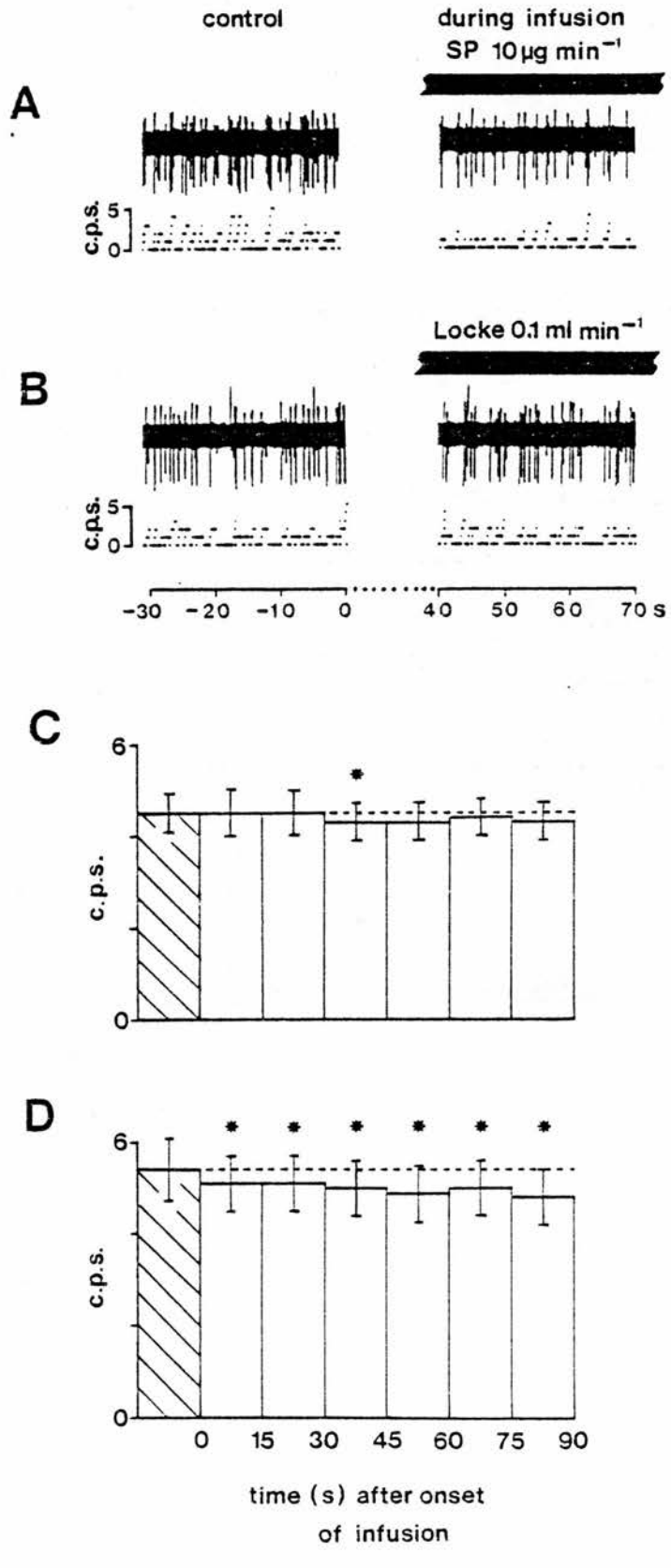
Chemodepression evoked by NA (0.1-10 µg; n=4) or DA (0.1-10 µg; n=4) was not markedly affected in any experiment during SP infusion, although there was a consistent tendency for the depressant effect of the highest doses injected to be potentiated, when compared with results obtained during Locke infusion (e.g. Fig 9.2 C,E). Chemodepression evoked by 5-HT (1-25 µg; n=4) was consistently enhanced during SP-infusion (cf. Fig. 9.2 D). Delayed excitatory reponses to NA and 5-HT were affected differently during peptide infusion. NA-evoked excitation (without distinction between E_1- and E_2- effects) was slightly reduced (see Fig 9.2 A), whilst secondary excitation evoked by 5-HT was clearly potentiated (e.g. Fig. 9.2 B). Initial transient excitation in response to 5-HT was not seen in these experiments.

Three-minute infusions of SP (10 µg min^{-1}) or Locke solution (0.1 ml min^{-1}) were made in four experiments during ventilation with 0, 2, 4, and 6% CO_2 in 15, 21 ('room air'), and 50% O_2, plus nitrogen. The average discharge at 1, 2, and 3 minutes after the onset of infusion at all levels of PaO_2 and $PaCO_2$ was not different when results obtained during SP infusion were compared with those obtained during

Figure 9.1. (A) and (B): recordings of 2-3 chemoreceptor units showing discharge before, and from 40 s after onset of a 2 min. infusion of (A) SP 10 μg min^{-1} i.c. or (B) Locke solution 0.1 ml min^{-1} i.c. A ramped counter output beneath each neurogram gives the number of action potentials counted in succesive 1 s intervals.

(C) Infusion of Locke solution (0.1 ml min^{-1}; n=63) caused a minimal reduction in chemoreceptor discharge apparent 30-40 s after onset of infusion. When SP (D) was infused (10 μg min^{-1}; n=49) there was a small but significant decrease ($P<0.05$; Student's t-test) in discharge, commencing within the first 15 s of infusion. The hatched blocks show the mean pre-infusion control discharge, and chemosensory discharge is expressed as mean (± s.e.m.) over 15 s intervals during a 90 s infusion period.

* = $P<0.05$ with respect to pre-injection control values.


control
during infusion
SP 10µg min⁻¹
A
c.p.s.
5
0
Locke 0.1 ml min⁻¹
B
c.p.s.
5
0
-30 -20 -10 0 40 50 60 70 s
C
6
c.p.s.
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D
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c.p.s.
0
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0 15 30 45 60 75 90
time (s) after onset
of infusion

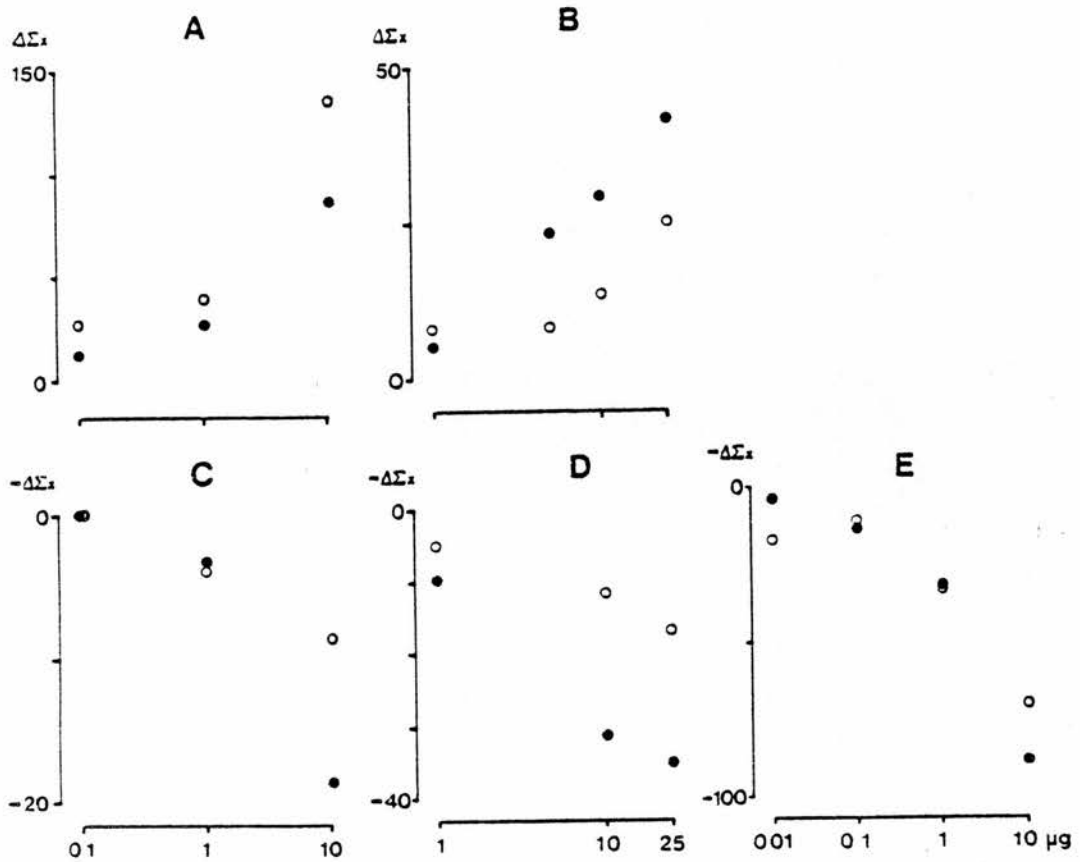


Figure 9.2. Results from single experiments showing the effects of SP infusion upon chemodepressant and excitatory effects evoked by injections of monoamines.

Integrated responses for the secondary chemoexcitation evoked by NA (A) and 5-HT (B) during Locke infusion (0.1 ml min^{-1} - open symbols) and during SP infusion (10 µg min^{-1} - closed symbols), showing a slight reduction of the effect of NA and a potentiation of the response to 5- HT during SP infusion.

Integrated responses for initial chemodepression evoked by NA (C) and 5-HT (D), and for the depressant response to DA (E) during Locke infusion (0.1 ml min^{-1} - open symbols) and during SP infusion (10 µg min^{-1} - closed symbols). Chemodepression evoked by 5-HT was potentiated during infusion of the peptide.

infusion of Locke solution.

9.2 Studies with vasoactive intestinal polypeptide.

9.2.1 Introduction and methods.

Experimental reports demonstrating the presence of VIP in the cat carotid body and the limited descriptions of its effects upon chemosensory afferent activity have been cited in Section 1. The limited availability of the peptide meant that only a few experiments could be performed. Experimental procedures were as described previously, and the possible interactions of monoamines and the peptide were investigated using the same methodology as in experiments with other peptides.

9.2.2 Results.

The effects of VIP were studied in two cats. Injection (i.c.) of 1 or 5 μg of the peptide caused variable but dose-related chemoexcitation in both experiments, with a latency of onset of less than 5 s (Fig. 9.3). There was a fall in systemic arterial blood pressure, but this commenced a few seconds after the increase in chemoreceptor discharge.

VIP infusions were carried out in both experiments. There was no obvious effect upon chemoreceptor discharge during infusion of VIP 1 μg min^{-1} i.c. for two minutes; when analysed in consecutive 15 s intervals, discharge was variously slightly increased or slightly reduced in comparison with the mean rate of discharge measured before the onset of infusion, and no consistent pattern of response was discernable, nor was there any correlation of these slight fluctuations in discharge with the time elapsed from onset of infusion. When

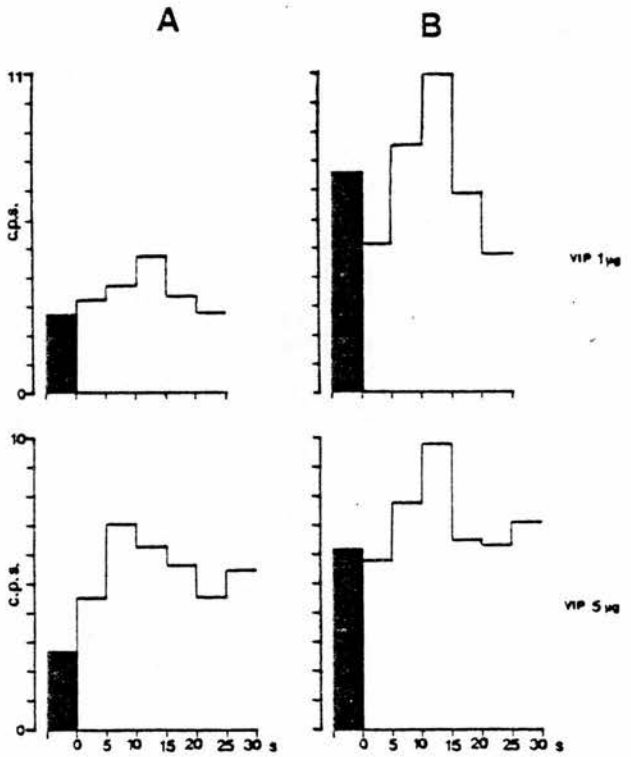


Figure 9.3. Effects of injection of VIP 1 or 5 µg i.c. (at t=0 s) upon chemoreceptor activity at low (A) and high (B) levels of background discharge, in the same experiment. Discharge frequency was averaged in successive 5 s intervals and plotted against time from injection. The mean discharge in the 15 seconds immediately prior to injection is shown by the solid block.

VIP was infused at 5 μg min^{-1} there was a clear hypotensive effect, but again no consistent effect upon chemoreceptor activity.

In both experiments dose-response curves were obtained for ACh (10-100 μg i.c.), DA (0.1-10 μg i.c.) and NA (1-25 μg i.c.), injected at 90 s of a two minute infusion of Locke solution (0.1 ml min^{-1}) or VIP (5 μg min^{-1}).

The integrated excitatory response ($\Delta\Sigma x$) to ACh was not markedly altered during VIP (Fig. 9.4A), but Δmax was approximately 20% higher at all doses during VIP infusion in one experiment. In the second experiment $\Delta\Sigma x$ was not obviously altered, and with high doses of ACh, Δmax was actually reduced during VIP infusion (Fig 9.4B).

Chemodepression evoked by DA was affected differently by VIP infusion in the two experiments. In one the integrated response and $(-)\Delta max$ were both increased, implying a slight enhancement of the depressant effect, whilst in the other experiment $\Delta\Sigma x$ was not markedly altered, and $(-)\Delta max$ was reduced (Fig. 9.5).

In the experiment in which the excitatory effect of ACh appeared to be potentiated, NA-evoked chemodepression and chemoexcitation were reduced during VIP infusion (Fig. 9.6A). In the second experiment NA-evoked chemodepression was not observed during VIP infusion (Fig 9.6B) whilst secondary E_2- (vascularly-mediated) chemoexcitation was reduced and E_1-excitation largely unaffected.

9.3 Summary of results presented in section 9.

1. Injection of SP or other tachykinins produced no consistent or clearly dose-related effect upon chemoreceptor discharge, save that depression preceded excitatory effects.
2. Infusions of SP were accompanied by a small but significant

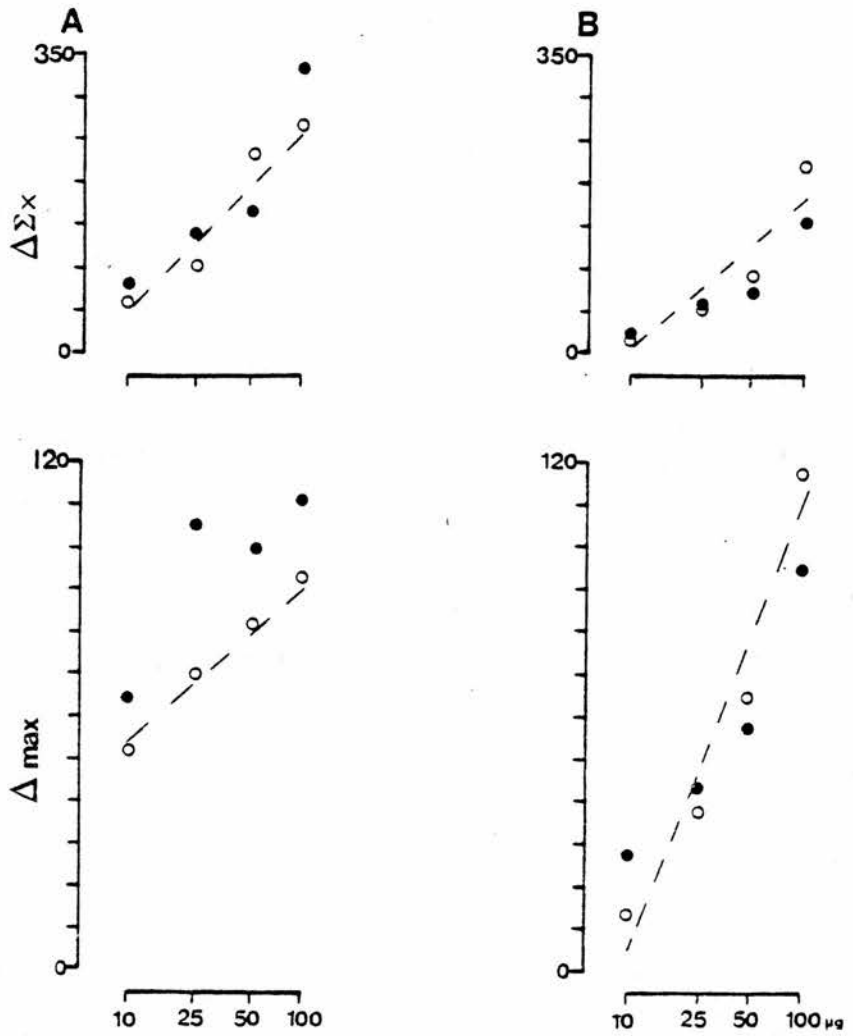


Figure 9.4. Effects of VIP infusion upon responses to ACh in two experiments, (A) and (B). The integrated response ($\Delta\Sigma x$) and Δmax quantifying the excitatory effects of ACh during Locke infusion (0.1 ml min^{-1} - open symbols) and during VIP infusion (5 µg min^{-1} - closed symbols) have been plotted against dose. In both experiments $\Delta\Sigma x$ was not markedly different during the two infusions, but in the experiment shown in (A) Δmax was increased by approximately 20% at all but one of the doses injected.

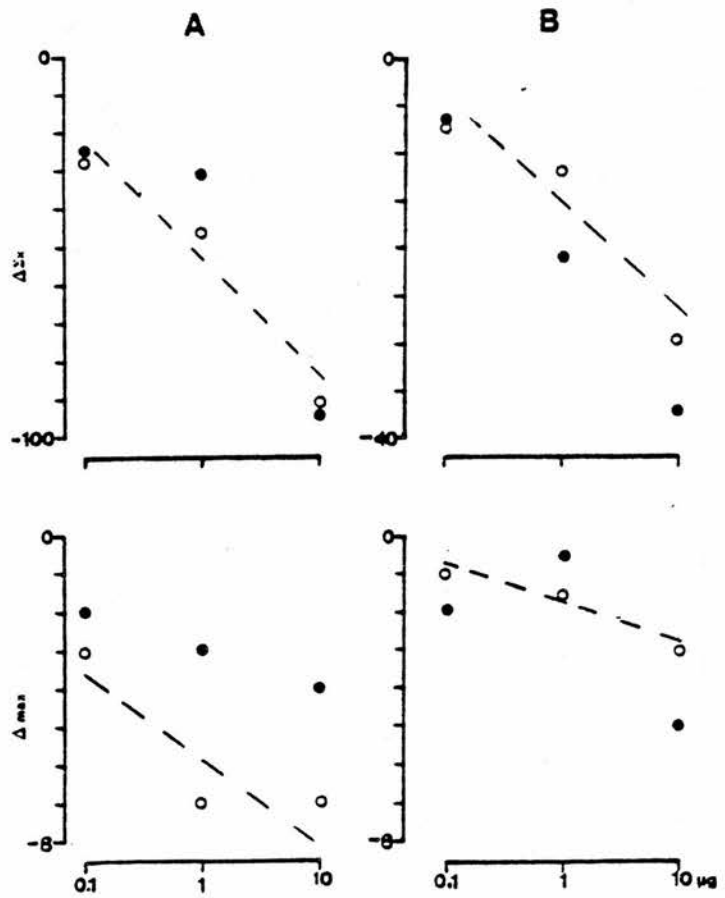
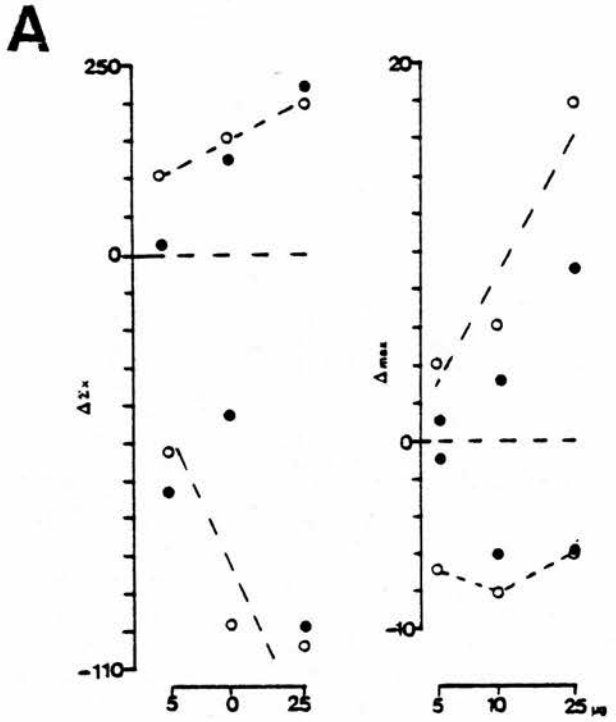


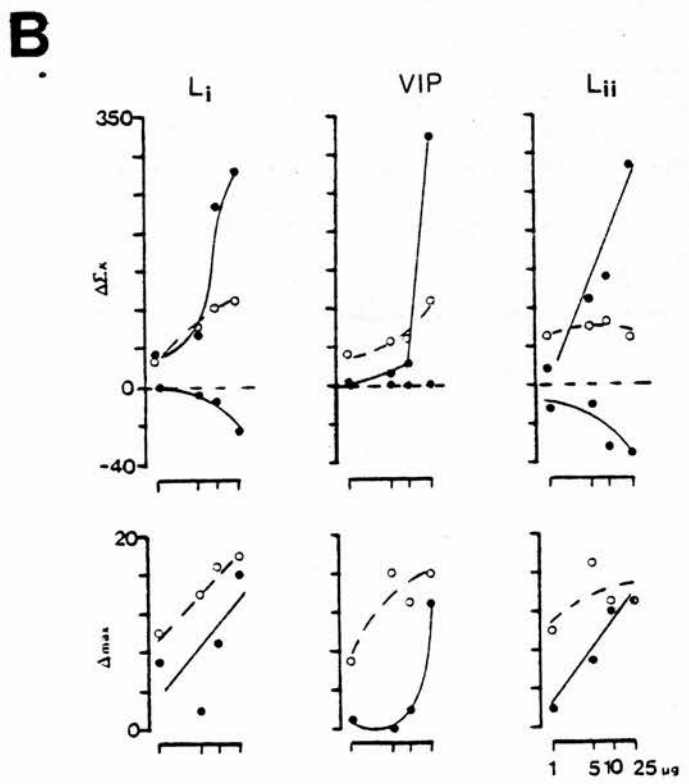
Figure 9.5. Effects of VIP infusion upon responses to DA in two experiments, (A) and (B). The integrated response ($\Delta\Sigma x$) and Δmax quantifying the chemodepressant effects of DA during Locke infusion (0.1 ml min^{-1} - open symbols) and during VIP infusion (5 µg min^{-1} - closed symbols) have been plotted against dose. The integrated response was not clearly altered during VIP infusions, but in the experiment shown in (A), (-)Δmax was reduced during infusion of the peptide.

Figure 9.6. Effects of VIP infusion upon responses to NA in two experiments, (A) and (B).

In (A) The integrated responses ($\Delta\Sigma x$) and Δmax quantifying the effects of NA during Locke infusion (0.1 ml min^{-1} - open symbols) and during VIP infusion (5 µg min^{-1} - closed symbols) have been plotted against dose. The integrated response was not clearly altered during VIP infusions, but Δmax and $(-)\Delta max$ were generally reduced during infusion of the peptide, implying a reduced responsiveness to the catecholamine.

In (B) The integrated responses ($\Delta\Sigma x$), and Δmax (for excitatory effects only) to NA during an initial Locke infusion (0.1 ml min^{-1} - Li), during VIP infusion (5 µg min^{-1} - VIP) and during a subsequent infusion of Locke solution (Lii) have been plotted against dose. For excitatory responses, E_1-excitation is represented by open symbols, and E_2-excitation by solid symbols. Chemodepression was not seen during the infusion of VIP, and both $\Delta\Sigma x$ and Δmax for the secondary or delayed E_2-excitatory effect were reduced during infusion of the peptide. E_1-excitation in response to NA was apparently unaltered by the peptide.


A
250
0
-110
ΔΣx
20
0
-10
Δmax
5
0
25
5
10
25 µg


B
Li
VIP
Lii
350
0
-40
ΔΣx
20
0
Δmax
1
5
10
25 µg

decrease in chemoreceptor discharge frequency, but responses to SP were unaltered during combined changes in PaO_2 and $PaCO_2$.

3. Chemodepressor responses to NA and DA were little affected by SP infusion; NA-evoked chemoexcitation was slightly reduced. Both initial chemodepression and delayed chemoexcitation evoked by 5-HT were potentiated during infusion of SP, this being the most consistent and most marked effect observed.

4. Bolus doses of VIP injected i.c. caused clear and relatively long-lasting chemoexcitation, together with hypotension. Infusion of the peptide at doses capable of reducing blood pressure had no clear effects upon rate of chemoreceptor discharge, which alternately rose and fell with no clear relationship to time from onset of infusion.

5. Chemoexcitation evoked by ACh was enhanced in one experiment and unaltered or reduced during VIP infusion in the other. DA-evoked chemodepression was enhanced in one experiment, and NA-evoked depression of discharge was absent during VIP infusion. In the other experiment DA- and NA- evoked chemodepression were both slightly reduced, although the effect was not marked. NA-evoked chemoexcitation during VIP infusion was reduced, although E_1-type excitation was largely unaffected, where distinguishable.

9.4 Discussion.

9.4.1 Effects of Substance P.

Interpretation of the effects of SP upon chemoreceptor activity is difficult because there was no consistent pattern of dose-related changes in activity, making it impossible to determine how long a time interval after injection of the peptide, or which part of this period,

should be studied. McQueen's data (1980b) do show a dose-related, if delayed, chemoexcitation in response to SP. In the present study the most consistent effect of SP was a small but statistically significant decrease in discharge frequency evoked during short infusions of SP, 10 μg min^{-1}.

It was suggested (McQueen, 1980b) that the observed excitatory response to SP may have been secondary to changes in $PaCO_2$ or cardiac output. Eyzaguirre et al (see Eyzaguirre & Fidone, 1980) reported a reduction in chemoreceptor discharge in response to SP in the cat, rabbit, and mouse carotid bodies in vitro, and more recently Monti-Bloch and Eyzaguirre (1985) reported that low doses of SP depressed, and higher doses excited the cat carotid body superfused in vitro; bolus doses of SP (43 ng - 0.5 μg) had similar effects in this preparation. Variations in results obtained with SP have also been seen, for example, in cultured mouse spinal neurones (Barker et al, 1980), so the ambivalent nature of results obtained in chemoreceptor studies is not unique.

None of the tachykinins tested in the present study proved more effective than SP, and this might indicate that it is a SP-P receptor rather than the SP-E sub-type that should be sought after. Evidence also suggests that there is no variation in the effect of SP at differing levels of PaO_2 and/or $PaCO_2$, although Monti-Bloch and Eyzaguirre (1985) did report that SP reduced the response to hypoxia in the in vitro preparation.

9.4.2 Interactions with monoamines.

The location of SP in fibres of the carotid sinus nerve (Cuello & McQueen, 1980) and the fact that exogenous SP depolarises type I

cells in vitro (see Monti-Bloch & Eyzaguirre, 1985), together with suggestions that the peptide may function as a transmitter in sensory afferent pathways projecting to the nucleus tractus solitarius (e.g. Gillis et al 1980; Helke et al, 1980a,b) suggests that SP could exert 'retrograde' effects upon the chemosensory mechanism. In this respect, the potentiation of the depressant and excitatory effects of 5-HT during SP infusion (also reported by McQueen - 1980b) may be of importance, particularly since the two have been shown to coexist in certain neurones (for references see Hökfelt et al, 1980).

McQueen (1980b) showed that DA-evoked chemodepression was reduced during SP infusion. In the present study the effect of DA was not obviously altered during SP infusion, although the depressant effect of 10 µg NA (perhaps higher doses could usefully have been studied) was consistently slightly potentiated during infusion of the peptide. This is more in accord with the potentiation of DA-evoked chemodepression in the in vitro study of Monti-Bloch and Eyzaguirre (1985).

The interactions of SP with monoamines gave qualitively better, more consistent results than the other experiments using this peptide. Chemodepression evoked by SP was much less intense than that observed during [MET]enk infusion (see section 8), and although NA-evoked chemoexcitation was consistently reduced during SP infusion, this is probably not accounted for by any marked reduction in background discharge (cf. Section 8), and the potentiation of responses to 5-HT also means that a reduction in rate of spontaneous discharge does not account for the altered actions of monoamines during infusion of SP.

Crucial to studies of the effects of SP in the carotid body would be the establishment of whether or not the peptide can here affect the

release or synthesis of transmitters such as DA or 5-HT, as it can in the CNS (e.g. Carlsson et al, 1977a; Reubi et al, 1978). Such studies are hampered by the fact that SP-systems appear to be particularly prone to desensitisation, and the lack of selective antagonists or drugs capable of selectively modulating the synthesis, release, and catabolism of SP does not allow the type of studies that have enabled advances to be made in the understanding of the actions of some of the other drugs used in this study.

9.4.3 Effects of vasoactive intestinal polypeptide.

The excitatory effect of injected VIP upon chemoreceptors reported by Fitzgerald et al (1981) and McQueen and Ribeiro (1981a) was confirmed in the present study. Chemoexcitation evoked by slow injection of VIP (5 or 25 μg) in the experiments reported by the former group did not display any marked dose-dependency, although the blood pressure effect was related to dose. As in the present experiments, the change in discharge frequency preceded changes in systemic arterial blood pressure. The fact that VIP is a large and complex molecule may mean that it does not easily reach or rapidly gain access to the chemosensory complex after i.c. injection, and perhaps a more active component of the molecule must be released by enzymatic cleavage. However, specific binding of VIP to VIP-receptors is held to be rapid, with achievement of 90% of maximum binding within 60 s in vitro (Makhlouf, 1985).

McQueen and Ribeiro reported sustained excitatory effects of infusions of VIP at amounts of as little as 0.5 μg min^{-1}, which were still obtained when the hypotensive effects of the peptide were countered by administration of dextran, suggesting that the response was

not a consequence of vascular effects of the peptide. In the present experiments no clear chemoexcitatory response to infusion could be discerned. In the earlier experiments (McQueen & Ribeiro, 1981a) a slight increase in discharge was apparent within the first minute of infusion, and the effect was considerably greater from the second minute onwards. In the present study infusions of VIP were made for two minutes only, but in both this and in the earlier set of experiments the infusion cannula was primed with polypeptide solution, so that there was no dead-space to clear at the beginning of the infusion. Furthermore, the vascular effects of infusing VIP 5 μg min^{-1} in the present experiments were very clear, and commenced within some 20-30 s of the onset of infusion. The dependence of the effects observed (either here or in the other sets of experiments) upon factors such as tissue penetration, association with or disocciation from receptors, activation of second messengers or, indeed, peptide activation or inactivation, is unknown.

9.4.4 Interactions with monoamines.

McQueen and Ribeiro (1981a) showed the effects of single doses of ACh (50 μg i.c.) to be reduced during VIP infusion. Results obtained in the present study do not confirm this, there being no clear change in the dose-response curve for ACh-evoked chemoexcitation in one experiment, and a clear potentiation of the effect in the other. It must be acknowledged that in the experiments of McQueen and Ribeiro there was more certainty that VIP was exerting effects upon the chemoreceptors at the time of injection. The present results are perhaps more akin to the synergistic activity of VIP and ACh in the feline salivary gland (Lundberg et al, 1980).

DA-evoked chemodepression (5 μg i.c.) was reduced during VIP infusion in the earlier experiments (McQueen & Ribeiro, 1981a), and there was no recovery after VIP infusion. In the current study VIP was only associated with a reduction of the depressant response to DA in one experiment. However, NA-evoked chemodepression (which appears to be mediated through the same receptors as the response to DA) was reduced during VIP infusion in both experiments (abolished in one), as was subsequent chemoexcitation (excluding E_1-excitation, which could be analysed independently in one experiment, and was unaffected by VIP infusion).

If VIP exerts actions in the carotid body through increases in intracellular cAMP levels (as in isolated gastric cells - Bitar & Makhlouf, 1982), then the interaction with D_2-receptors (perhaps negatively linked to adenylate cyclase) and with β-adrenoceptors (positively linked to the enzyme) will depend largely upon the relative amounts of peptide or monoamines injected (or reaching their respective receptors, or, indeed, the chemosensory complex), and the temporal relationship of the delivery of the different types of neurotransmitter.

Clearly, further studies of the effects of these two peptides must be performed before a definite role in chemoreception can be proposed or precluded for either. It might appear that the capacity of SP to alter chemoreceptor responses to other transmitters, particularly 5-HT may be of greater importance than its own rather weak and inconsistent effects upon the chemoreceptors.

SECTION 10

GENERAL CONCLUSIONS

SECTION 10.

GENERAL CONCLUSIONS.

This study represents an attempt to determine the pharmacology of the effects upon carotid chemoreceptor discharge of exogenous administration of several different classes of neurotransmitters which have been located within the carotid body. Such information will prove useful in defining the possible role of these substances contained in the organ.

The assumptions made were that monoamines such as NA, DA, 5-HT and ACh could act as typical neurotransmitters at an early stage in the mechanism of chemotransduction; polypeptides such as enkephalins, SP and VIP may also act as 'discrete' transmitters in defined pathways, but could also act more generally as 'neurohormones', possibly modulating the responsiveness of target cells to other transmitters.

Quantification of the chemoreceptor response is desirable so as to establish whether an effect is dose related, and to enable comparisons to be made between individual animals, or before and after selective antagonists. It was, however, one of the more difficult aspects of this study. Changes in Δmax evoked by NA were usually a constant function of dose, but after DA-blockade with domperidone this parameter appeared to be dose-related. This might support the idea that DA normally suppresses spontaneous activity (e.g. Osborne & Butler, 1975). In the case of excitatory responses to NA an obvious contrast exists between the integrated response ($\Delta\Sigma x$) and the difference in mean discharge (pre-injection control and post-drug excitation). The mean increase in discharge evoked by NA did not

generally vary with dose, and with NA or SAL, where the mean discharge in the period of drug-evoked excitation did increase, background (control) discharge appeared to be increased by the same amount. Integration of either excitatory or depressant responses proved the best or only means of producing a measure of the dose-dependency of the observed changes in chemoreceptor activity in response to injections of catecholamine-related agonists, but it is probably wise to use this measurement in comparison with an assessment of maximal or mean discharge frequency, since, in the case of NA, $\Delta\Sigma x$ appears to further support the correlation of catecholamine-evoked excitation with the duration of the systemic effects.

When comparing depressor responses to NA and DA both were seen to cause a complete suppression of discharge, hence $\Delta\bar{x}\%$ was often of the order of 90-100% for all doses. Since duration of the effect is shorter with NA, integration allowed a more meaningful comparison of the effects of these two drugs. This may be compared with depressor responses to 5-HT (see Section 7) where the depressor response was rarely -100% of control and $\Delta\bar{x}\%$ was more obviously correlated with dose. In certain circumstances $\Delta\bar{x}\%$ is meaningful if restricted to a particular time interval, but this appears to depend upon the property of chemoreceptors to respond minimally at low doses, so that depression of discharge in, for instance, the first five seconds (cf. effects of 5-HT - Section 7) or the first thirty seconds (cf. effects of opioid peptides - Section 8) after injection ranges from threshold (≥ 0) towards 100% or maximum, as dose is increased. With NA such analysis was not feasible since chemodepression was rather an 'all-or-none' event, being complete, but of variable duration, thus tending always to represent a 100% depression of chemoreceptor activity. It is

not meaningful with complex responses to extend the duration of this period to accomodate the time-dependency of higher doses since the onset of excitation confounds the analysis. Similarly, with excitatory responses that follow depressant effects, it is not possible to standardise quantification in this way since the time of onset cannot be accurately related to the time of injection. The interphase between chemodepression and chemoexcitation must represent a particularly complex dynamic state of neural and biochemical activity. Data obtained during infusions of drugs are clearly of value because the effect of a drug may be studied when steady-state conditions prevail.

Several important conclusions may be drawn from this work. With respect to the actions of NA, evidence has been provided that this catecholamine could function essentially as an 'inhibitory' substance, causing a decrease in the frequency of spontaneous discharge. This action is almost certainly mediated through the activation of DA D_2-receptors within the carotid body. That this is the primary action of NA is suggested not only by the rapid onset of the effect, but also by the fact that chemodepression is maintained during the infusion of the drug, even when cardiovascular changes (which appear to underly the delayed chemoexcitation following injections of NA) are as marked as those evoked by injections of NA.

Vasoconstrictive-hypertension almost certainly does <u>not</u> directly cause the increase in chemoreceptor discharge following injection of NA (as evidenced by the failure of the α_1-selective agonist PHEN to cause chemoexcitation) unless accompanied by other changes which alter (reduce) cardiac output or otherwise result in changes in arterial blood gas tensions.

A transient chemoexcitatory response to NA was also observed in some 67% of recordings; this effect was not susceptible to blockade with adrenoceptor antagonists (nor to blockade with cholinoceptor antagonists), and it remains to be established whether it is mediated through atypical adrenoceptors. The biochemical mechanisms that subserve this transient excitatory response are yet to be determined. There is a strong possibility that the effect is the same as that observed by O'Regan (cf. O'Regan, 1983) in response to sympathetic nerve stimulation. The significance of this effect in terms of 'continuous' chemoreceptive activity is uncertain.

The pattern of results obtained suggests some similarities with the DA-receptor mechanism characterised in the intermediate lobe of the rat pituitary gland. In this tissue enhanced formation of cAMP, in response to β-adrenoceptor stimulation by ISO, results in release of α-melanocyte stimulating hormone; both responses are inhibited by DA, acting through D_2-receptors (Kebabian & Calne, 1979). Apomorphine too causes a non-competitive decrease in the maximum response of the β-adrenoceptor to ISO, whereas PROP is a competitive antagonist at the β-adrenoceptor (see Kebabian & Cote, 1981). NA is able to stimulate both the β-adrenoceptor in the intermediate lobe of the pituitary (causing an increase in cAMP levels - Cote et al, 1980) and the DA D_2-receptor (see Kebabian & Cote, 1981). Responses to NA are smaller than those to ISO, but are potentiated after the DA-antagonist fluphenazine, which does not alter the response to ISO. The suggested mechanism for the observed effects of NA in the pituitary model is that stimulation of the D_2-receptor causes a non-competitive reduction in the responsiveness of the β-adrenoceptor, resulting in a sub-maximal enhancement of adenylate cyclase activity (Kebabian & Cote, 1981).

Such a mechanism could account for the effects of NA upon chemoreceptor discharge observed in the present study, and the sustained depressant effect of NA during infusions of the drug implies that D_2-receptor activation might indeed be a mechanism for attenuating chemoreceptor discharge. This accords with theories that tonic release of DA exerts a 'braking' effect upon chemoreceptor discharge. Evidence from the present study suggests that β-adrenoceptors are not important in the intrinsic proccesses of chemotransduction, and perhaps other candidates (amino acids or purines?) must be investigated as the 'primary' transmitters involved in chemotransduction, if, indeed, the response to physiological stimulation is mediated by the release of transmitter substances.

The role of 5-HT contained in the carotid body still remains to be determined. The use of novel antagonists has allowed a tentative classification of receptors involved in the complex response to 5-HT. Thus, initial transient chemoexcitation, observed in about half the recordings, and the more consistent chemodepression evoked by 5-HT, are blocked by the antagonist MDL 72222. This novel antagonist 'selectively' blocks the effects of 5-HT at 'neuronal' receptor sites, which, together with the rapid onset of these effects of 5-HT is suggestive of a direct action upon the sensory nerve endings, or at a site close to a mechanism inducing depolarisation of these nerves. The less intense, delayed chemoexcitatory responses to 5-HT (which initially appear to coincide with vascular changes evoked by the drug) are blocked by the 5-HT_2 antagonist ketanserin, but it was also observed that this type of response was more prominent after administration of the antagonist MDL. It is possible that the effect is mediated by a less-sensitive 5-HT-receptor in the carotid body,

which, like the proposed excitatory DA-receptors (see Section 1) can only be seen to cause changes in chemoreceptor discharge after the effects of stimulating more-sensitive receptors have been abolished. How such a desensitisation would occur in vivo is unclear, but it could be a potentially useful homeostatic mechanism.

The failure of blockade of 5-HT receptors or of adrenoceptors to prevent the response of chemoreceptors to hypoxia suggests that neither indole- nor catechol- amines are involved in the intrinsic mechanism of hypoxic chemotransduction.

Opioid peptides, namely the enkephalins, cause chemodepression, and the results of this pharmacological study indicate that the effect is mediated through the δ-sub-type of opioid receptor.

Chemodepression caused by NA and by [Met]enk is capable of reducing the response of chemoreceptors to hypoxia (and in the case of the opioid at least, to hypercapnia). The physiological stimuli applied in these experiments were relatively severe; possibly, transient release of these substances in the carotid body could 'smooth out' the effects of momentary fluctuations of gas tensions in the blood supplying the carotid body. Such an action might also be synergistic with efferently controlled regulation of blood flow, or direct efferent control of nerve activity (cf. Section 1), and the whole subject of efferent control of the carotid body has yet to be fully investigated.

Least conclusive are the effects of the polypeptides SP and VIP. The former had no readily definable action upon the chemoreceptors, whilst the latter was a chemoexcitant, and might be seen potentially to complement the actions of the opioids. This must remain speculative until circumstances for its release in the carotid

body are determined.

The interaction of polypeptides with monoamines deserves further consideration. In this respect, the most consistent and striking observation was the potentiation of chemodepression and subsequent excitation in response to 5-HT during infusion of SP. This is consistent with reports of only slight alterations of membrane potential by SP (see Section 1), followed by facilitated responses to other neurotransmitters.

Since it appeared that the depressant effects of DA and NA were not amplified during infusion of [Met]enk, this (as previously suggested - Section 8) may indicate that both classes of neurotransmitter act through (inhibition of?) a common (cAMP?) mechanism.

All forms of response to NA, DA, and ACh were potentiated during NAL infusion, and after the potent δ-selective antagonist ICI 174864, the response to hypoxia was also potentiated. This suggests that endogenous opioids tonically suppress spontaneous chemoreceptor activity, as proposed by Pokorski and Lahiri (1981), and an action also proposed for DA (Osborne & Butler, 1975; McDonald & Mitchell, 1975a; Krammer, 1978). It is unclear why both DA and opioids should mediate the same homeostatic effect, unless the dynamics of the actions they exert are different, thus allowing the possibility of 'coarse' and 'fine' tuning of regulatory actions.

In this study no attempt was made to evaluate the effects of monoamines upon the responsiveness of chemoreceptors to peptides, and this allows scope for future studies. Of critical importance is the careful balancing of the amounts of drugs applied in these in vivo pharmacological studies, so as not to mask subtle effects of one drug by gross effects of another. The marked depressant effects of [Met]enk

seen in the present study may well account for the equivocal nature of the results obtained in the study of interactions with monoamines.

Catechol- and indole- amines and opioid peptides depress chemoreceptor activity; SP may also partially depress chemoreceptor activity. None of the drugs studied would appear to be a likely candidate as an 'essential' excitatory transmitter in the proccesses of chemotransduction; most of these drugs appear more capable of acting as depressors, thus modulating spontaneous activity. Clearly, if released substances are to be held accountable for modulating spontaneous activity in a way meaningful to the accepted excitatory effects of physiological stimulation, then much more information must be obtained concerning both the evoked release of these substances, and the possible role of the efferent nerves in causing their release. In this respect, it would be necessary to establish what form of 'dialogue' takes place between centres in the brain stem that receive an afferent input from the chemoreceptors, and the centres from which efferent activity is initiated.

Two problems must be solved in order to determine how the carotid body chemoreceptors function. First, the identity of the structure within the carotid body, responsible for detection of changes in PaO_2 and $PaCO_2$ must be identified. The type I cells may be necessary to obtain chemosensory activity on a moment-to-moment basis, and could do this by release of a transmitter, triggering activity in the sensory nerve endings. They may also, or instead, be essential for chemoreception through the production of some trophic factor(s) which, on a long term basis may induce chemosensory properties in the nerve endings.

The second problem concerns the establishment of what biochemical or biophysical changes in the transducer mechanism are caused by the

stimulus. Here, the studies directed towards the 'metabolic' effects of O_2 may suggest how changes in PaO_2 are sensed, and the use of techniques to study changes in membrane potential (e.g. Zapata & Eyzaguirre, 1985) will become increasingly more important. All the drugs and stimuli that have been applied to the carotid body to evoke changes in afferent neural activity will have to be reapplied to determine their effects upon membrane potentials and resistances, trans-membrane ionic currents, respiratory enzyme chains, intracellular Ca^{2+} homeostasis and so forth. The effects upon neural activity of the drugs used in the present study may be useful in interpreting effects upon these other parameters, but it should always be borne in mind that exogenously administered transmitter substances may act at sites not normally affected by the same substances, contained or released within the carotid body.

The enigma of this tiny organ, about which volumes have been written, will continue to engage scientists and theoreticians for many a year to come.

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ACKNOWLEDGEMENTS

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APPENDIX 1

DETAILS AND SOURCES OF DRUGS USED IN THIS INVESTIGATION

APPENDIX 1: DETAILS AND SOURCES OF DRUGS USED IN THIS INVESTIGATION.

(Doses referred to in the text are those of the salt).

Drug	M.Wt.	Source
Acetylcholine iodide	273.1	B.D.H.
[D-Ala^2,D-Leu^5]enkephalin	569.7	C.R.B.
[D-Ala^2,MePhe^4,Glyol^5]enkephalin	514.6	C.R.B.
Betaxolol hydrochloride	343.9	Synthélabo, Paris
α-Chloralose	309.5	B.D.H.
Corynanthine hydrochloride	390.9	Sigma
Dobutamine hydrochloride	337.8	Lilly
Domperidone	425.9	Janssen, Belgium
Dopamine hydrochloride	189.6	Koch Light
Dynorphin$_{(1-8)}$ octapeptide	981.3	C.R.B.
Eledoisin	1188.6	Penninsula Labs
Ethylketocyclazocine methanesulphonate	403.1	Sterling Winthrop
Gallamine triethiodide ('Flaxedil')		May & Baker
8-Hydroxy-DPAT hydrobromide	328.3	Merrell, France
5-Hydroxytryptamine, creatinine sulphate complex	387.4	Sigma
ICI 118,551 hydrochloride	339.5	I.C.I.
ICI 154,129 arginate	922.0	I.C.I.
ICI 174,864 base	715.0	I.C.I.
Isoprenaline sulphate	556.6	Pharmacy Dept., Royal Inf. Edin.
Kassinin	1334.7	Peninsula Labs
Ketanserin tartrate	545.5	Janssen, Belgium
[Leucine^5]enkephalin acetate	555.7	C.R.B.
MDL 72222 methanesulphonate	419.3	Merrell, France
[Methionine^5]enkephalin acetate	573.7	C.R.B.
5-Methoxy-tryptamine hydrochloride	226.7	Sigma
2-Methyl 5-hydroxytryptamine, creatinine sulphate complex	401.4	Glaxo
Metoprolol tartrate	684.8	Ciba Geigy
Morphiceptin [β-casomorphin$_{(1-4)}$amide] hydrochloride	558.1	Sigma
(-)-Naloxone hydrochloride	363.8	Sigma
L-Noradrenaline bitartrate	337.3	Sigma

Drug	M.Wt.	Source
Oxymetazoline hydrochloride	296.5	Merck Sharp & Dohme
L-Phenylephrine hydrochloride	203.7	Koch Light
Physalaemin	1265.6	Peninsula Labs
Prenalterol hydrochloride	261.7	Astra
DL-Propranolol hydrochloride	295.8	I.C.I.
Rauwolscine base (α-yohimbine)	354.4	Inverni della Beffa, Milan, Italy
RU 24969 succinate	346.1	Roussel, France
Salbutamol base	239.3	Allen & Hanburys
Sodium cyanide	49.0	B.D.H.
Sodium pentobarbitone ('Sagatal')		May & Baker
Substance K (neurokinin α)	1133.5	C.R.B.
Substance P	1347.7	C.R.B.
Substance P, methyl ester	1364.8	C.R.B.
L-Sulpiride	341.4	Ravizza, Italy
Vasoactive intestinal polypeptide $_{(1-28)}$	3326.3	C.R.B.

Common reagents for the preparation of Locke solution etc. were obtained commercially, and were of 'analar' grade.

APPENDIX 2

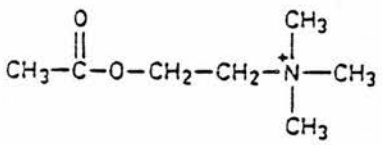
MOLECULAR STRUCTURES OF AGONISTS & ANTAGONISTS USED IN THIS INVESTIGATION

APPENDIX 2: MOLECULAR STRUCTURES OF AGONISTS & ANTAGONISTS USED IN THIS INVESTIGATION.

(The receptor type at which a substance is expected to exhibit activity is indicated in parentheses.)

1. MISCELLANEOUS CHEMORECEPTOR STIMULANTS.

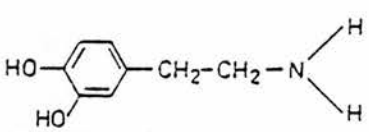
Sodium cyanide



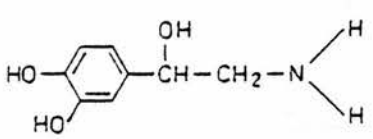
Acetylcholine

2. DRUGS USED IN STUDIES ON THE EFFECTS OF CATECHOLAMINES.

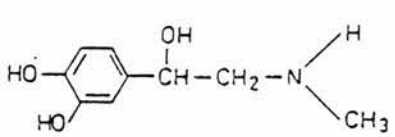
2.1 Endogenous catecholamines.



Dopamine

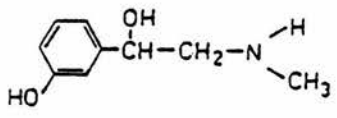


Noradrenaline ($\alpha_1/\alpha_2/\beta_1$)

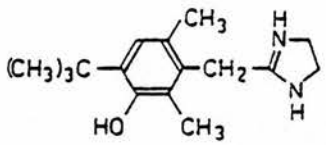


Adrenaline ($\alpha_1/\alpha_2/\beta_1/\beta_2$)

2.2 Selective α - Adrenoceptor agonists.

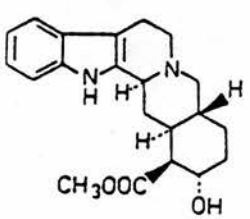


Phenylephrine (α_1)

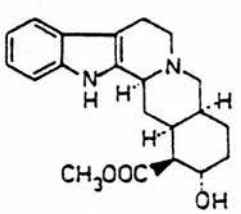


Oxymetazoline (α_2)

2.3 Selective α - Adrenoceptor antagonists.

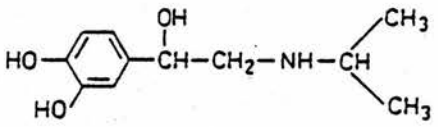


Corynanthine (α_1)

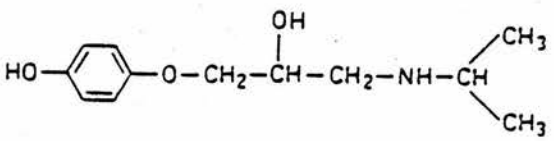


Rauwolscine (α_2)

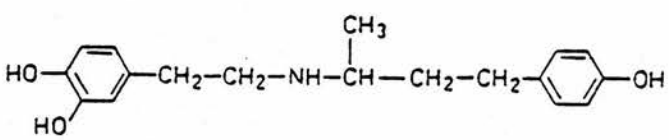
2.4 Selective β - Adrenoceptor agonists.



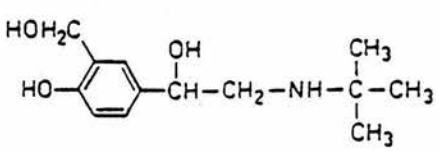
Isoprenaline (β_1/β_2)



Prenalterol (β_1)

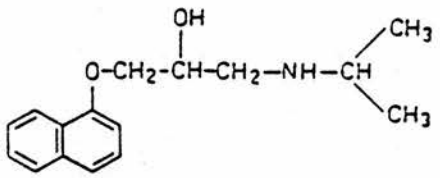


Dobutamine (β_1)

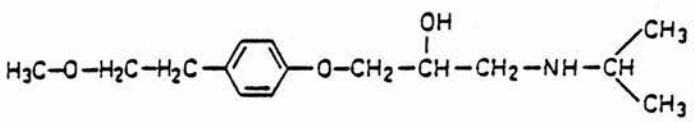


Salbutamol (β_2)

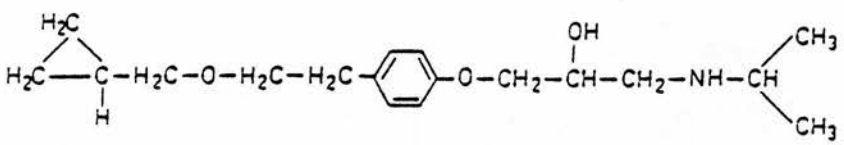
2.5 Selective β - Adrenoceptor antagonists.



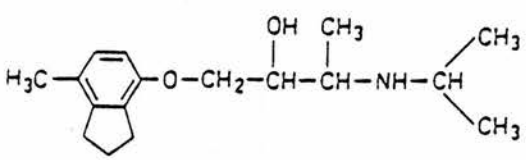
Propranolol (β_1/β_2)



Metoprolol (β_1)

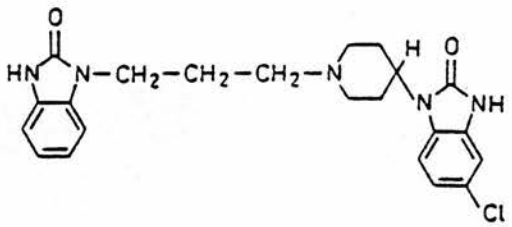


Betaxolol (β_1)

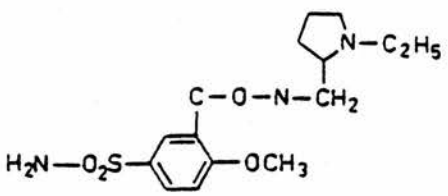


ICI 118,551 (β_2)

2.6 Dopamine antagonists.



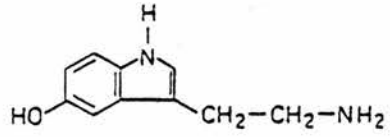
Domperidone (D_2)



Sulpiride (D_2)

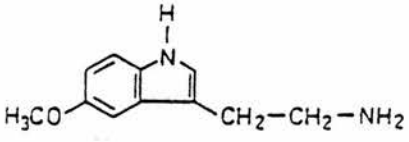
3. DRUGS USED IN STUDIES ON THE EFFECTS OF 5-HT.

3.1 Endogenous ligands.

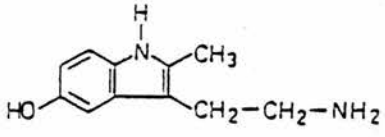


5-Hydroxytryptamine

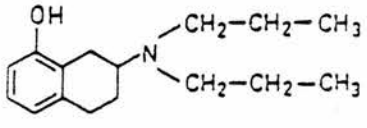
3.2 Selective 5-HT agonists.



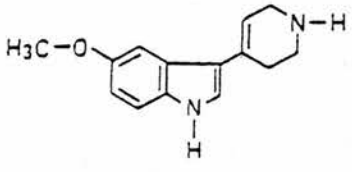
5-Methoxytryptamine
(inactive at MDL - sensitive sites)



2-Methyl, 5-hydroxytryptamine
(MDL - sensitive sites)

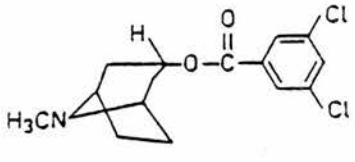


8-OH DPAT (5-HT_{1A})

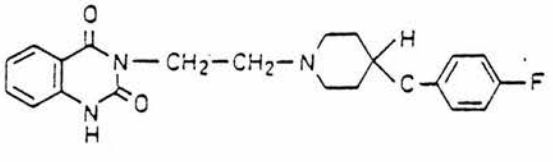


RU 24969 (5-HT_{1B})

3.3 Selective 5-HT Antagonists.



MDL 72222
(neuronal 'M' sites)



Ketanserin (5-HT_{2})

4. TACHYKINININS.

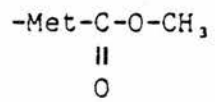
4.1 Substance P and related compounds.

Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
Eledoisin	PCA-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH_2
Kassinin	Asp-Val-Pro-Lys-Ser-Asp-Gln-Phe-Val-Gly-Leu-Met-NH_2
Physalaemin	PCA-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH_2
Substance K	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2

(All the tachykinins display approximately equal potency at the 'SP-P' receptor, whilst the 'SP-E' receptor is considerably more sensitive to eledoisin and kassinin than to the other tachykinins.)

4.2 Synthetic Substance P analogues.

The methyl ester of Substance P is formed by the substitution of a methyl group on the terminal methionine residue by means of an ester linkage:



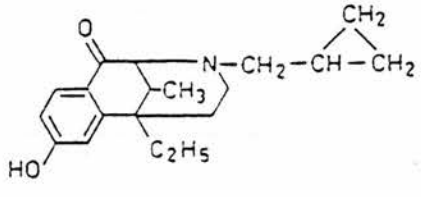
5 OPIOID PEPTIDES.

5.1 Endogenous opioid peptides.

Methionine Enkephalin ($\delta > \mu >>> \kappa$)	Tyr-Gly-Gly-Phe-Met-NH_2
Leucine Enkephalin ($\delta >> \mu >>> \kappa$)	Tyr-Gly-Gly-Phe-Leu-NH_2
Dynorphin$_{(1-8)}$ octapeptide ($\kappa > \mu > \delta$)	Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-NH_2

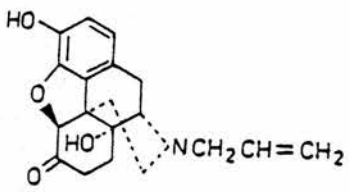
5.2 Substituted enkephalins & other non-endogenous opioid agonists.

[DADL]enkephalin ($\delta > \mu >> \kappa$)	Tyr-D-Ala-Gly-Phe-D-Leu-NH_2
[DAGO]enkephalin ($\mu >> \delta, \kappa$)	Tyr-D-Ala-Gly-MePhe-$NH(CH_2)_2OH$
Morphiceptin [β-casomorphin$_{(1-4)}$ amide] ($\mu >>> \delta$)	Tyr-Pro-Phe-Pro-NH_2

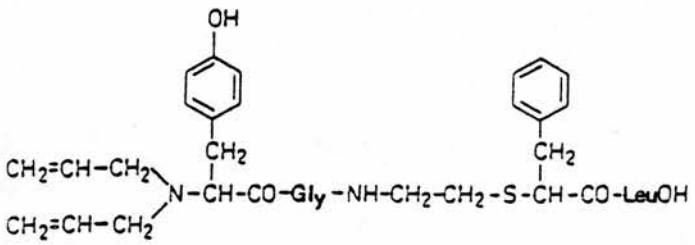


Ethylketocyclazocine ($\kappa(>\mu>\delta)$)

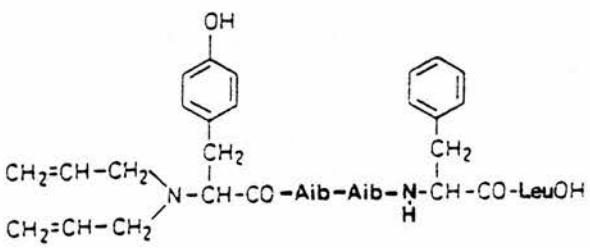
5.3 Opioid antagonists.



Naloxone (μ>κ>δ)



ICI 154,129 (δ>>μ,κ)



ICI 174,864 (δ)

6 Vasoactive Intestinal Polypeptide.

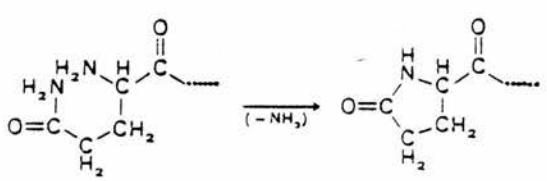
His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr
Lys-Val-Ala-Met-Gln-Lys-Arg-Leu-Arg-Thr
Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2

Key to amino acids:

Ala: Alanine	His: Histidine	Ser: Serine
Arg: Arginine	Ile: Isoleucine	Thr: Threonine
Asn: Asparagine	Leu: Leucine	Trp: Tryptophane
Asp: Aspartic acid	Lys: Lysine	Tyr: Tyrosine
Gln: Glutamine	Met: Methionine	Val: Valine
Glu: Glutamic acid	Phe: Phenylalanine	
Gly: Glycine	Pro: Proline	

Aib = α-aminoisobutyric acid

PCA = Pyrrolidine carboxylic acid, the cyclic derivative of a terminal glutamic acid residue.



Glutamic acid PCA

APPENDIX 3

PUBLICATIONS

[*From the Proceedings of the Physiological Society*, 23–24 *September* 1983
Journal of Physiology, **346**, 96*P*]

Changes in responses of cat carotid body chemoreceptors to 5-HT after administration of the antagonist MDL 72222

By G. C. Kirby and D. S. McQueen. *Department of Pharmacology, University of Edinburgh Medical School, Edinburgh EH8 9JZ*

The physiological role of the 5-hydroxytryptamine (5-HT) that is present in the cat carotid body (Chiocchio, Biscardi & Tramezzani, 1967) has yet to be established. Nishi (1975) found that intracarotid (I.C.) injection of 5-HT in cats caused a transient increase in carotid chemoreceptor discharge that was followed by a period of chemodepression. These responses were unaffected by the putative 5-HT antagonists LSD, gramine and methysergide.

In the present study we have investigated the effects of MDL 72222 (a new compound which appears to be a selective antagonist at neuronal 5-HT receptors – Fozard, 1983) on the responses of carotid-body chemoreceptors to 5-HT, dopamine and to changes in P_{a,O_2}. Experiments were performed on cats anaesthetized with α-chloralose (70 mg kg^{-1} I.V.) artificially ventilated and paralysed with gallamine (3 mg kg^{-1} I.V.). Chemoreceptor activity was recorded from the peripheral end of a carotid sinus nerve, and drugs were injected into the common carotid artery, as previously described (Docherty & McQueen, 1978).

Injection of 5-HT (0·1–100 μg I.C.) caused a dose-dependent depression of 'spontaneous' chemoreceptor discharge which was followed by a delayed excitation of variable duration. Only occasionally was the chemodepression preceded by a transient burst of activity, in agreement with the findings of Black, Comroe & Jacobs (1972). After MDL 72222 the dose–response curve for 5-HT-evoked chemodepression was shifted to the right, and following the higher dose of antagonist 5-HT caused a prolonged dose-dependent chemo-excitation of rapid onset. The initial transient burst of activity, when present, was also abolished by the antagonist. Chemodepression evoked by dopamine and chemo-excitation caused by sodium cyanide were unaffected by the doses of MDL 72222 studied, and there were no marked changes in spontaneous discharge or, as judged from preliminary experiments, in the response of the chemoreceptors to alterations in P_{a,O_2}.

Our results show that MDL 72222 is capable of antagonizing the depressant effect of 5-HT on carotid chemosensory discharge and may prove useful in helping to determine the physiological role for 5-HT in the carotid body.

This work is supported by a project grant from the M.R.C. G.C.K. is a Houldsworth Scholar of Edinburgh University. MDL 72222 was kindly donated by Merrell (Strasbourg).

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Black, A. M. S., Comroe, J. H. & Jacobs, L. (1972). *Am. J. Physiol.* **223**, 1097–1102.
Chiocchio, S. R., Biscardi, A. M. & Tramezzani, J. H. (1967). *Science, N.Y.* **158**, 790–791.
Docherty, R. J. & McQueen, D. S. (1978). *J. Physiol.* **279**, 425–436.
Fozard, J. R. (1983). Proceedings of British Pharmacological Society Meeting, July 1983. (In the Press.)
Nishi, K. (1975). *Br. J. Pharmac.* **55**, 27–40.

Br. J. Pharmac. (1984), **83**, 259–269

Effects of the antagonists MDL 72222 and ketanserin on responses of cat carotid body chemoreceptors to 5-hydroxytryptamine

G.C. Kirby & D.S. McQueen

Department of Pharmacology, University of Edinburgh Medical School, Edinburgh EH8 9JZ

1 The effects of intracarotid (i.c.) injections of 5-hydroxytryptamine (5-HT; 1–50 μg) on carotid chemoreceptor activity recorded from the carotid sinus nerve have been studied in anaesthetized cats.

2 Three separate components in the complex response of the chemoreceptors to injected 5-HT were identified. Firstly, a transient burst of activity was obtained during the injection period in 56% of the recordings. Secondly, in all the recordings a period of chemodepression commenced a few seconds after completing the injection and was usually dose-related. Thirdly, a delayed longer-lasting chemoexcitation occurred in many experiments, concomitant with a fall in systemic blood pressure.

3 The neuronal 5-HT receptor antagonist MDL 72222 (10–100 $\mu g\,kg^{-1}$, i.c.) virtually abolished the transient chemoexcitation evoked during 5-HT injections and also significantly increased the mean ID_{50} for 5-HT-induced chemodepression; in 37% of recordings 5-HT caused a dose-related chemoexcitation after the high dose of MDL 72222. Neither the delayed chemoexcitation nor the hypotension caused by 5-HT were much affected by the antagonist. MDL 72222 itself had a biphasic effect on chemosensory discharge, causing depression followed by a delayed excitation.

4 The $5\text{-}HT_2$-receptor antagonist ketanserin (100 $\mu g\,kg^{-1}$, i.c.) had no appreciable effect on the transient chemoexcitation evoked during 5-HT injections and caused a slight but significant increase in the mean ID_{50} for 5-HT-induced chemodepression. The delayed chemoexcitation and accompanying hypotension associated with 5-HT were both substantially reduced or abolished by the antagonist. Ketanserin itself caused a short-lasting period of chemoexcitation.

5 All the effects of injected 5-HT on chemosensory discharge could be abolished by the combination of MDL 72222 and ketanserin (100 $\mu g\,kg^{-1}$, i.c.).

6 Neither MDL 72222 nor ketanserin had any significant effect upon the response of the carotid chemoreceptors to hypoxia. The rate at which discharge increased, and also the steady-state discharge before and during hypoxia, were unaffected by the antagonists, alone or in combination.

7 At least two types of 5-HT receptor appeared to be involved in the response of carotid body chemoreceptors to 5-HT. Transient excitation and chemodepression were mediated via MDL 72222-sensitive (peripheral neuronal) receptors whereas the delayed chemoexcitation and associated hypotension involved a ketanserin-sensitive, presumably $5\text{-}HT_2$-, receptor. It appears unlikely that 5-HT plays a crucial role in chemoreception.

Introduction

Although it is well known that 5-hydroxytryptamine (5-HT, serotonin) is present in the carotid body of many species, including the cat (e.g. Chiocchio *et al.*, 1967), its physiological role there remains to be determined. The effects of exogenous 5-HT on chemosensory activity recorded from the carotid sinus nerve have been studied in anaesthetized cats (Black *et al.*, 1972; Nishi, 1975; Docherty & McQueen, 1978). A complex response is obtained following the intracarotid injection of 5-HT, with a common pattern being a brief period of chemoexcitation followed by a longer-lasting depression of background chemoreceptor discharge. Nishi (1975) found that the response to 5-HT was unaffected by

atropine or hexamethonium, which eliminates involvement of acetylcholine (ACh) receptors, but the putative 5-HT antagonists lysergic acid diethylamide (LSD), gramine and methysergide were also without effect on the response of the carotid chemoreceptors to 5-HT. Categorization of 5-HT receptors in the peripheral nervous system is a complex problem (see Gyermek, 1961; Wallis, 1981) and the value of studies with drugs such as methysergide or LSD in helping to characterize the carotid body 5-HT receptors has been questionable because they lack specificity as 5-HT antagonists. However, the recent advent of more specific 5-HT antagonists such as MDL 72222, shown to be potent in antagonizing the actions of 5-HT at peripheral neuronal sites (e.g. fibres mediating the Bezold-Jarisch reflex in rat – Fozard, 1984), and ketanserin, which is reputed to be a highly selective antagonist at 5-HT_2-receptor sites but inactive at 5-HT_1-receptor sites (Leysen *et al.*, 1981), prompted us to study their effects on the cat carotid chemoreceptors. The aim was to attempt a characterization of 5-HT receptors involved in the response of cat carotid chemoreceptors to 5-HT and, by investigating the influence of the antagonists on responses of the chemoreceptors to a physiological stimulus, hypoxia, obtain information regarding the physiological role of 5-HT in the carotid body.

A preliminary account of some of the results has previously been presented (Kirby & McQueen, 1984).

Methods

Experiments were performed on fifteen cats of either sex, weighing between 2.3 and 3.9 kg, median weight 3.0 kg. Animals were anaesthetized with α-chloralose (65–70 mg kg^{-1}, intravenously) following induction with halothane (5% in oxygen) and supplements of chloralose were administered intravenously as required. In two experiments pentobarbitone (42 mg kg^{-1}, intraperitoneally) was used instead of α-chloralose.

Full details of the experimental procedures have been given previously (McQueen, 1977; Docherty & McQueen, 1978) so only a brief description is provided here. The carotid sinus region on one side was dissected and the ganglioglomerular nerves, which carry the sympathetic nerve supply from the superior cervical ganglion to the carotid body, were cut. The cats were artificially ventilated with room air, apart from periods when hypoxic gas mixtures were used. Gallamine triethiodide (3 mg kg^{-1}) was administered intravenously to prevent spontaneous and drug-induced muscle movements. Drugs were dissolved in modified Locke solution or in 0.9% w/v NaCl solution (saline) and injected, in volumes of 0.1 ml, into the common carotid artery ipsilateral to the sinus nerve from which electrical activity was recorded. They were washed in with 0.2 ml Locke solution which had been bubbled with 5% CO_2:95% air at 37°C; injections were generally completed within 1–2 s.

Electrical activity of single or multiple (2–4) chemoreceptor units was recorded from the peripheral cut end of the sinus nerve and stored on tape for subsequent analysis using a pulse height voltage discriminator linked to a microcomputer (McQueen *et al.*, 1984). The units were confirmed as chemoreceptors by their random pattern of discharge, their increase in discharge frequency following injection of sodium cyanide (2.5 μg) into the ipsilateral common carotid artery or in response to hypoxia (breathing 10% oxygen in nitrogen), and by the depression of discharge in response to hyperoxia (breathing 100% oxygen).

Data analysis

A plot of chemosensory discharge (counts per 0.1 s bin) against time was made for each test, and the change in discharge frequency ($\bar{x}$ c.p.s.) from the pre-injection control frequency calculated. In order to standardize results from experiments with different absolute discharge frequencies, the response occurring in the first 5 s of the post-injection period was expressed as a percentage change from control level and plotted against $\log_{10}$ dose. From lines fitted to the dose-response data it was possible to calculate the dose causing a particular response (e.g. ID_{50}, dose causing a 50% reduction in control discharge) and obtain a mean value by pooling data from different experiments.

Hypoxia

The animals were made hypoxic by switching the inspired gas from air to 10% O_2 plus 90% N_2 for 4 min. For each test the control (air-breathing) discharge and steady-state discharge (hypoxia) were measured and arterial blood samples taken before and 3.5 min after onset of the hypoxic stimulus for blood gas analysis. Discharge was measured over consecutive 15 s periods and plotted against time, and a straight line fitted to the values obtained when discharge was increasing in response to the hypoxic stimulus (i.e. until a steady-state maximum (100%) or plateau discharge was obtained). The slope of this line provided an index of the rate of increase in chemoreceptor discharge in response to hypoxia, and was expressed as % max s^{-1}.

Statistics

Mean values are given ± s.e.mean. Statistical analysis of differences between means was carried out using the Wilcoxon two-sample test and the null hypothesis rejected at the 0.05 level of probability (2-tailed).

Drugs

The following compounds were used, and doses are expressed in terms of the salt: 5-hydroxytryptamine creatinine sulphate complex, dopamine hydrochloride (Sigma); MDL 72222 (1αH, 3α, 5αH-tropan-3-yl 3,5-dichlorobenzoate) methanesulphonate salt, kindly donated by Merrell International, Strasbourg; domperidone and ketanserin, tartaric acid salt, kindly donated by Janssen Pharmaceuticals, Beerse, Belgium.

Results

5-HT injections

Sixteen recordings of chemosensory activity were obtained, and intracarotid injection of 5-HT (1–50 μg) consistently caused a *depression* of chemosensory discharge which lasted for 3–15 s. The effect was dose-related in twelve of the recordings (75%), as shown in Figure 1. In the other four experiments chemodepression was not clearly related to dose. 5-HT doses of less than 1 μg had only slight effects on background discharge and these did not differ significantly from those associated with injection of the drug vehicle; the latter had variable effects on chemosensory discharge causing, on average, a 20.5% reduction during the 5 s post-injection period. In nine recordings (56%) chemodepression

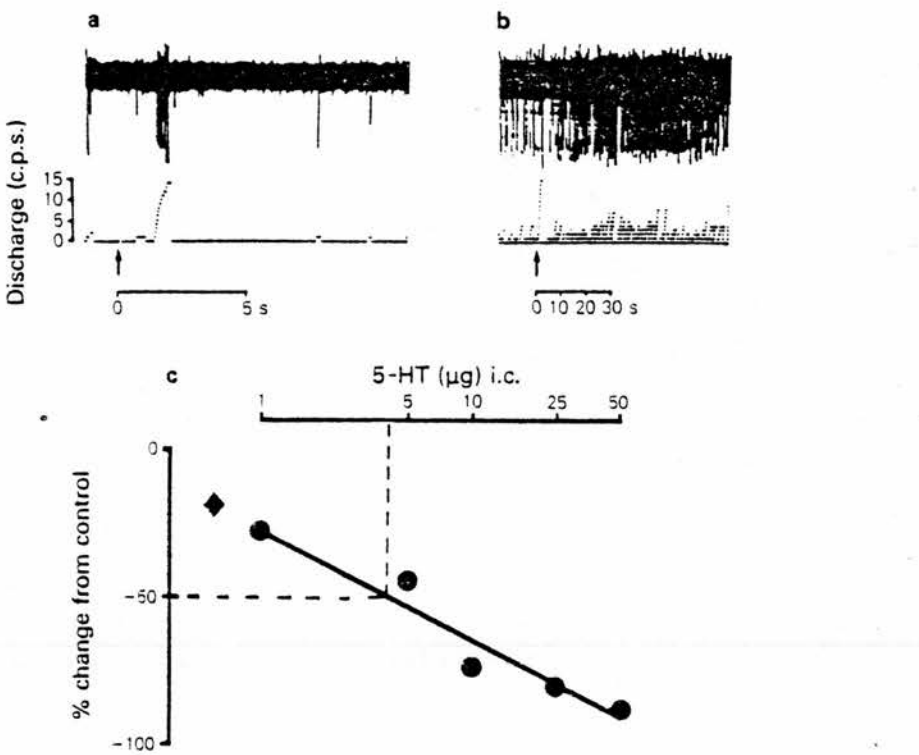

Figure 1 (a and b) Neurograms showing the effects of a single intracarotid (i.c.) injection of 5-hydroxytryptamine (5-HT; 10 μg, at arrow) on chemosensory discharge (3 units) and illustrating in (a) the initial burst of activity which is followed by a period of relative inhibition. A delayed phase of excitation can be seen in (b), which shows the same test displayed at a slower sweep speed. A ramped output below the neurograms gives the number of action potentials counted cumulatively in successive 1 s intervals. (c) Dose-response data showing the relationship between 5-HT dose and the percentage change in chemosensory discharge (from pre-injection control levels) that occurred during the first 5 s after injecting 5-HT. The straight line was fitted to the data by the least squares method, and the ID_{50} (i.e. dose of 5-HT causing a 50% depression of discharge – see broken lines) determined. The mean of the five individual control discharge values, from which the percentage changes were calculated, was 8.03 ± 0.26 c.p.s.. (◆) Effect associated with injection of the drug vehicle.

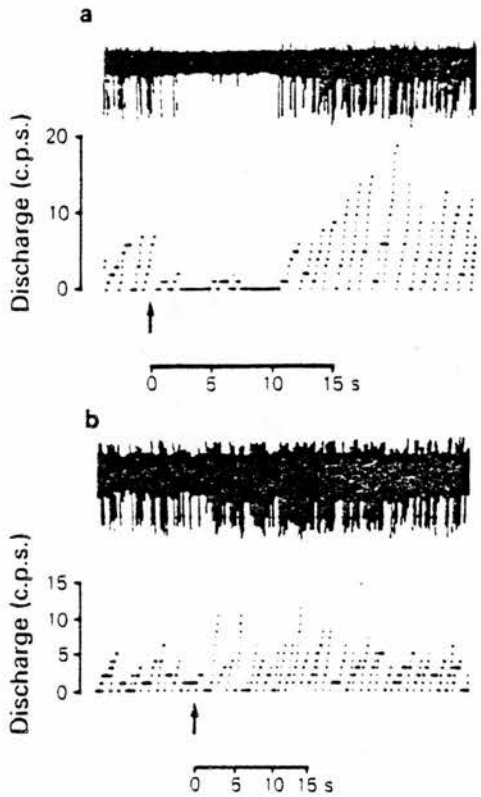

Figure 2 Neurograms of chemosensory discharge illustrating the response to injecting, at the arrow, (a) MDL 72222 (100 $\mu g\,kg^{-1}$, i.c.) and, from a separate experiment, (b) ketanserin (100 $\mu g\,kg^{-1}$, i.c.). The ramped trace below each neurogram gives the number of action potentials counted cumulatively in 1 s intervals.

was preceded by a transient burst of chemoreceptor action potentials, usually occurring within the injection period (Figure 1). The threshold for this more variable *excitatory* effect ($\approx 10\,\mu g$) was generally higher than that for chemodepression, but although discharge increased substantially, by 800–1000% in some experiments, a clear dose-response relationship was obtained in only three of the experiments, and the response appeared to be subject to tachyphylaxis. In the remaining 25% of recordings in which 5-HT did not cause transient excitation, chemodepression was still obtained. The averaged ID_{50} for the dose-dependent chemodepression in the recordings where the effect was dose-related was $5.8 \pm 1.9\,\mu g$ ($n = 12$).

In many of the experiments a *delayed increase* in chemosensory discharge was observed following the chemodepression (Figure 1). This effect was rather variable, lasted for 10–60 s, and had no consistent dose-response relationship. It appeared to be associated with the fall in systemic blood pressure that occurred following 5-HT injection (Figure 5).

Effects of the antagonist MDL 72222

Intracarotid injection of MDL 72222 (10–100 $\mu g\,kg^{-1}$) caused a depression of background chemosensory discharge which was followed by a delayed increase in discharge frequency (Figure 2a). No chemoexcitation was observed during the period of injection, and systemic blood pressure was not significantly affected by the antagonist. MDL 72222 (10 $\mu g\,kg^{-1}$, i.c.) was studied on seven recordings and in each case the dose-response line relating dose of

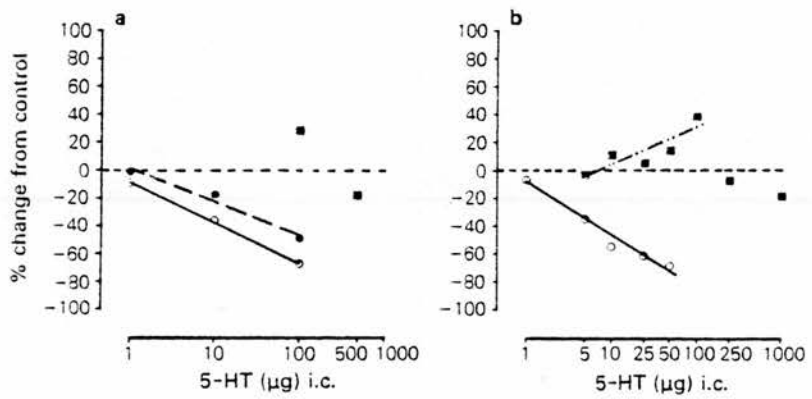

Figure 3 (a) Chemodepressant effect of 5-hydroxytryptamine (5-HT) injected before (○——○) and after (●----●) a low dose of MDL 72222 (10 $\mu g\,kg^{-1}$, i.c.). The rightward shift in the $\log_{10}$ dose-response curve caused by the antagonist is shown. An additional dose of MDL 72222 (100 $\mu g\,kg^{-1}$, i.c.) caused a further shift upwards and to the right (■). (b) In a separate experiment the higher dose of MDL 72222 (100 $\mu g\,kg^{-1}$, i.c.) completely abolished the 5-HT-induced chemodepression (■-···-■) and slight chemoexcitation was obtained in response to the lower doses of 5-HT. Lines were fitted to the data by the method of least squares.

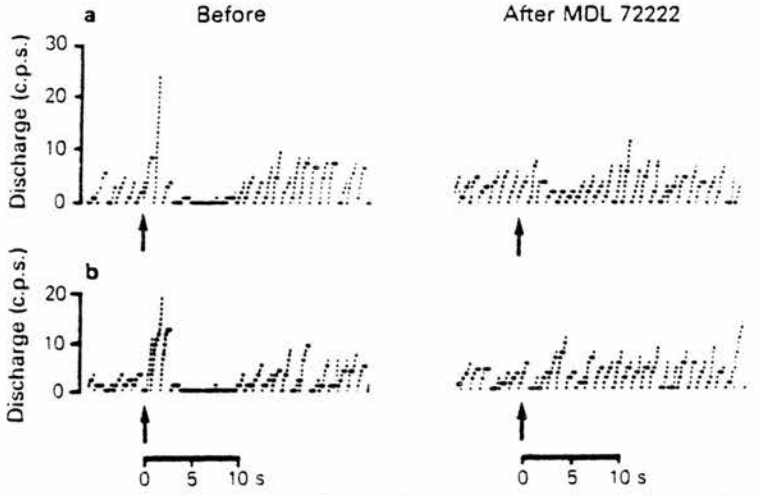

Figure 4 Responses of chemoreceptors (multi-unit recordings) to intracarotid injections of 5-hydroxytryptamine ((a) 5 and (b) 25 μg at arrows) before and after MDL 72222 (100 μg kg^{-1}, i.c.). The initial transient chemoexcitation and the subsequent depression of chemoreceptor discharge were both virtually abolished by the antagonist, and chemoexcitation became evident within the 5 s post-injection period. The ramps show the number of action potentials counted cumulatively in 1 s intervals.

5-HT to chemodepression was shifted upwards and to the right (Figure 3), and the mean ID_{50} was increased to 49.4 ± 33.6 μg ($n = 7$; $P < 0.05$ with respect to controls). When a dose of 100 μg kg^{-1} was given (8 recordings, in 6 of which it followed the lower dose) the ID_{50} increased to 638 ± 408 μg in five (63%) of the recordings. In the other three cases (37%) a dose-dependent chemoexcitation was obtained in the 5 s post-injection period (Figure 3), and no chemodepression occurred unless very high doses of 5-HT (250–1000 μg) were injected. It was possible to calculate an ED_{30} (dose causing 30% increase in discharge above pre-injection level), and this was 103.1 ± 9.0 μg ($n = 3$).

During the nine experiments in which 5-HT caused an initial transient excitation, this part of the response was substantially reduced (totally abolished in two recordings – 22%), by the lower dose of

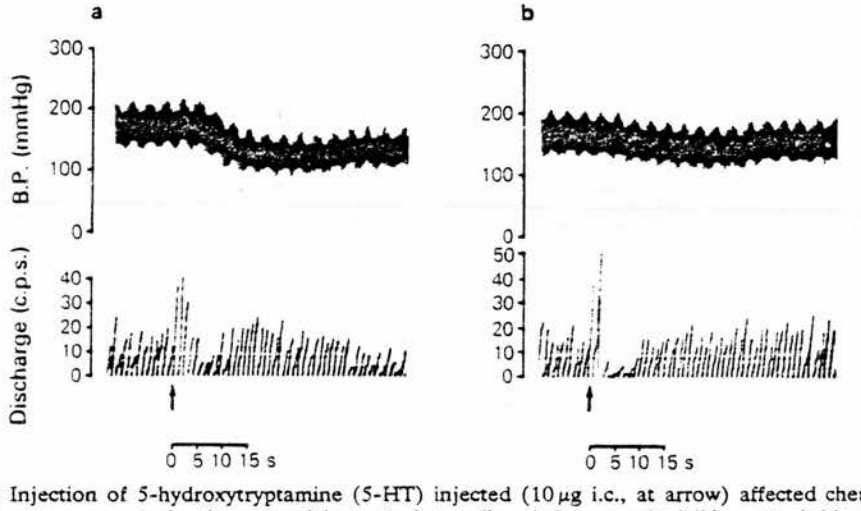

Figure 5 (a) Injection of 5-hydroxytryptamine (5-HT) injected (10 μg i.c., at arrow) affected chemosensory discharge (lower trace, count of action potentials per 1 s interval) and also caused a fall in systemic blood pressure (upper trace). (b) After administering ketanserin (100 μg kg^{-1}, i.c.) the same dose of 5-HT had much less effect upon blood pressure and there was less delayed chemoexcitation 10–15 s after the injection. However, both the initial burst of activity and the subsequent chemodepression were relatively unaffected by the antagonist.

MDL 72222 ($10\,\mu g\,kg^{-1}$), and further reduced or abolished by the higher dose ($100\,\mu g\,kg^{-1}$) – see Figure 4. In one experiment the 5-HT-induced transient chemoexcitation appeared only after MDL 72222 ($10\,\mu g\,kg^{-1}$) and was abolished by adding the higher dose ($100\,\mu g\,kg^{-1}$) of antagonist. The delayed or secondary chemoexcitation, which was more obvious following higher doses of 5-HT, generally increased in magnitude and became more rapid in onset after MDL 72222 ($10-100\,\mu g\,kg^{-1}$), as shown in Figure 6a.

Effects of the antagonist ketanserin

Intracarotid injection of ketanserin ($100\,\mu g\,kg^{-1}$) increased chemosensory discharge in the five recordings studied, an effect which lasted for 10–30 s (Figure 2b), and caused a longer-lasting fall in systemic blood pressure. There was no depression of discharge following ketanserin and no transient chemoexcitation during the injection period. The initial excitation caused by 5-HT was present in three of these recordings and was unaffected by the antagonist (Figure 5). The ID_{50} for 5-HT-induced chemodepression was $14.2 \pm 5.0\,\mu g$ ($n = 4$), which represents a small but significant ($P < 0.05$) decrease in the average response after ketanserin – although much less marked than the antagonism caused by MDL 72222 ($10\,\mu g\,kg^{-1}$), and ketanserin had little or no influence in some of the tests (e.g. Figure 5). In all five recordings the delayed increase in discharge was substantially reduced, as was the hypotensive effect of 5-HT (Figure 5).

Effects of the two antagonists in combination

In five experiments where MDL 72222 ($100\,\mu g\,kg^{-1}$) was administered after ketanserin ($100\,\mu g\,kg^{-1}$) the normal responses to 5-HT injections were absent. Dose-response data for the first 5 s of the responses gave lines of such shallow slope that meaningful ID_{50} or ED_{30} values could only be obtained by extrapolation far beyond the range of doses that could feasibly be used in the experiments, and these were not considered to be meaningful. This was also the case in the five experiments where ketanserin ($100\,\mu g\,kg^{-1}$) was injected after MDL 72222 ($100\,\mu g\,kg^{-1}$).

Responses to dopamine, and the effects of domperidone

The chemodepressant effect of dopamine (0.1–10 μg, i.c.) was obtained in all 16 recordings and was unaffected by either ketanserin or MDL 72222 (Figure 6). The dopamine D_2-receptor antagonist domperidone ($10-100\,\mu g\,kg$, i.c.$^{-1}$) was injected in

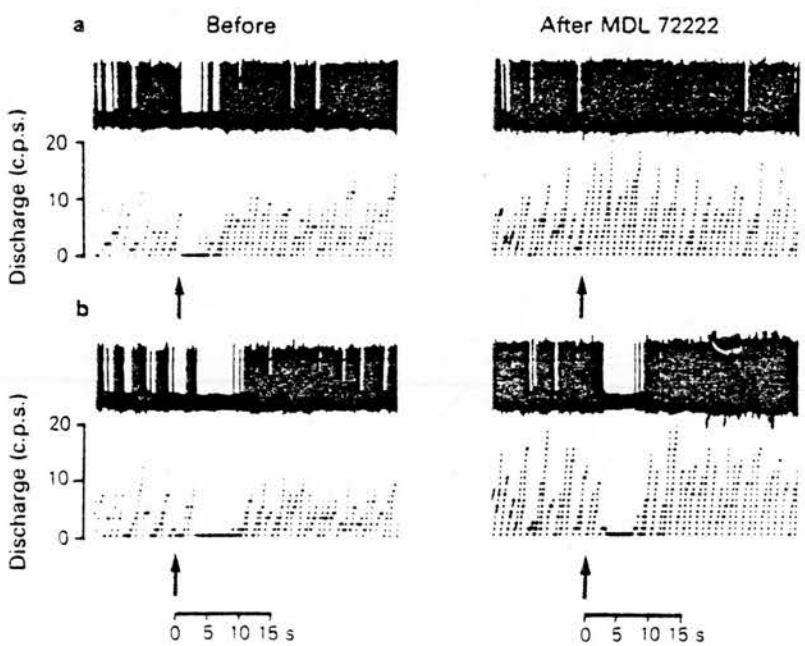

Figure 6 Neurograms showing the response of a single chemoreceptor unit to injections (arrows) of (a) 5-hydroxytryptamine 25 μg, i.c.) and (b) dopamine (1 μg, i.c.) before and after administering MDL 72222 ($100\,\mu g\,kg^{-1}$, i.c.). It can be seen that, whereas the chemodepression evoked by 5-HT was greatly reduced by the antagonist, the dopamine-induced effect was unaltered. The ramps show the number of action potentials counted cumulatively in 1 s intervals.

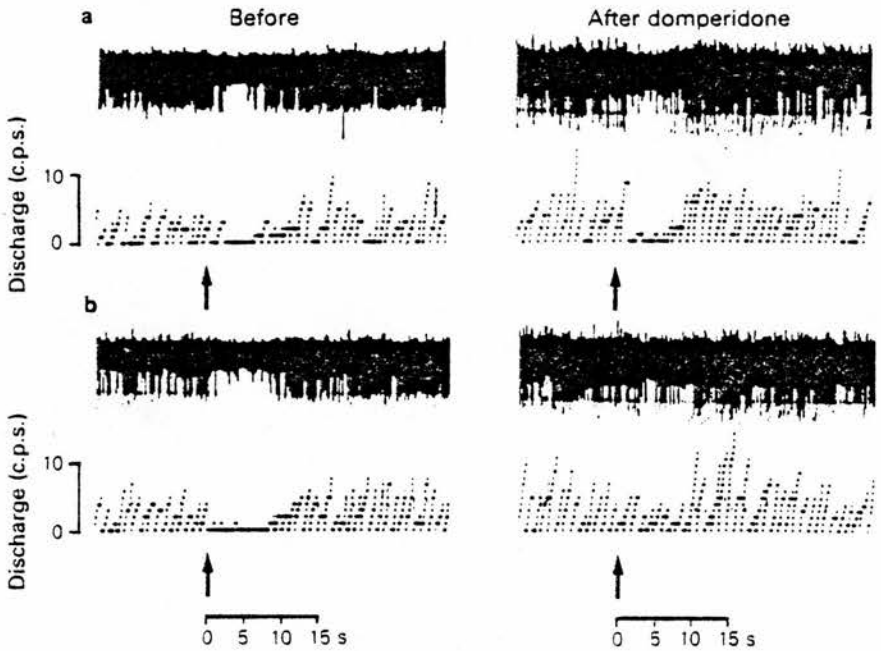

Figure 7 Neurograms showing the responses of chemoreceptor units to injections (arrows) of (a) 5-hydroxytryptamine 10 μg, i.c.) and (b) dopamine (1 μg, i.c.) before and after administering the dopamine antagonist domperidone (10 μg kg^{-1}, i.c.). The antagonist virtually abolished the chemodepressant effect of dopamine but had no appreciable effect upon the response evoked by 5-HT. The ramps show the number of action potentials counted cumulatively in 1 s intervals.

eight preparations and reduced the depressant effect of dopamine on the chemoreceptors without altering the responses to 5-HT (Figure 7), whether injected before (4 experiments) or after (4 experiments) the 5-HT antagonist(s). ID_{50} values for 5-HT-induced chemodepression obtained after domperidone alone were 10.8 ± 2.5 μg ($n = 4$) and 8.1 ± 3.1 μg ($n = 2$) for the 10 and 100 μg kg^{-1} doses, respectively ($P > 0.05$ in comparison with controls).

Responses to physiological (hypoxic) stimulation

The effect of hypoxic stimulation was studied in 14 experiments, and chemoreceptor responses obtained before and after administering MDL 72222 or ketanserin.

MDL 72222 Control chemosensory discharge frequency averaged 6.8 ± 1.5 c.p.s. on air, and increased to 26.8 ± 5.3 c.p.s. on 10% O_2 ($n = 6$). After MDL 72222 (10 μg kg^{-1}) background discharge on air increased to 10.8 ± 3.2 c.p.s., and rose to 27.3 ± 5.8 c.p.s. ($n = 6$) on 10% O_2. The slope of the line relating discharge to time during the period of increasing discharge was 1.08 ± 0.11% max s^{-1} before, and 1.04 ± 0.12 after MDL 72222. The time taken to reach the plateau or steady state discharge (i.e. maximum or 100%) was 112 ± 6 s before, and 109 ± 11 s after the low dose of MDL. A higher dose of MDL 72222 (100 μg kg^{-1}) was studied in 12 experiments (see Figure 8) in three of which ketanserin had previously been injected. Background discharge on air was 5.3 ± 1.0 c.p.s., and it increased to 21.9 ± 3.2 c.p.s. on 10% O_2, the slope being 1.02 ± 0.07% max s^{-1} and the time taken to reach plateau (max) 114 ± 4 s. After MDL 72222 background discharge was 7.7 ± 1.8 c.p.s., discharge on 10% O_2 26.3 ± 5.5, slope 1.08 ± 0.11, and the time taken to reach plateau 116 ± 7 s. None of these parameters was significantly different from control values, following either dose of MDL 72222.

Ketanserin In 6 hypoxia tests performed immediately before the injection of ketanserin (in one of which MDL 72222 had been administered previously) background activity in the pre-ketanserin control state was 6.9 ± 2.0 c.p.s., and increased to 25.6 ± 6.5 c.p.s. on 10% O_2, the slope of the line being 0.99 ± 0.06% max s^{-1}, reaching the plateau or maximum value 112 ± 6 s after changing gases. Following ketanserin (100 μg kg^{-1}) five hypoxia tests were carried out, and there was no significant change

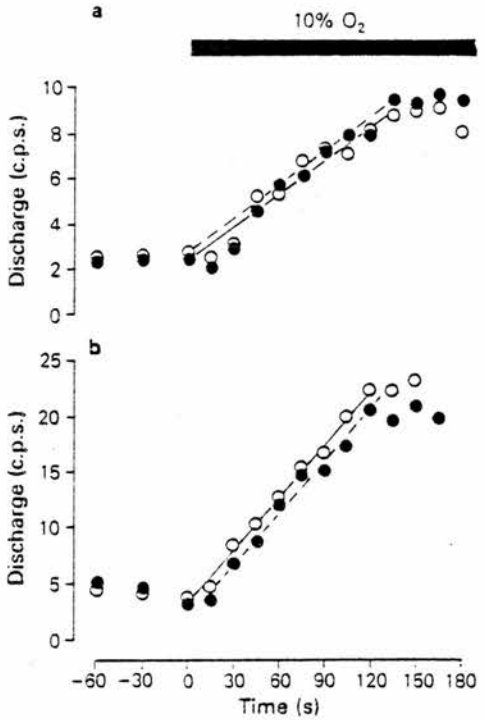

Figure 8 Increase in chemoreceptor discharge caused by ventilating the animals with a hypoxic gas mixture (10% O_2:90% N_2 during the 4 min period starting at 0 s, black bar) instead of room air. (a) Shows the discharge averaged over 15 s intervals, obtained before (○——○) and after (●---●) administering ketanserin (100 $\mu g\,kg^{-1}$, i.c.). (b) Shows the responses obtained, from a separate experiment, before (○——○) and after (●---●) administering MDL 72222 (100 $\mu g\,kg^{-1}$, i.c.). Lines were fitted to the data in the ranges shown by using the least squares method. Neither of the antagonists had any significant effect upon the response to hypoxia.

in any of the parameters (Figure 8). Background discharge was 6.9 ± 2.0 c.p.s., increasing to 26.0 ± 9.5 during 10% O_2, the slope was 1.02 ± 0.14% max s^{-1}, and time to plateau was 116 ± 17 s.

The mean values of arterial blood gas tensions and pH during air breathing (Pa_{O_2} 12.7 ± 0.7 kPa; Pa_{CO_2} 4.3 ± 0.1 kPa; pH 7.30 ± 0.01, $n = 17$) showed no significant differences between control values and those obtained after the administration of antagonists. Similarly, when the animals were made hypoxic, there were no significant differences in blood gas tensions and pH between the values obtained before (Pa_{O_2} 4.8 ± 0.2 kPa; Pa_{CO_2} 4.1 ± 0.2 kPa; pH 7.32 ± 0.01, $n = 17$) and after administration of the antagonists.

Discussion

The present results show that 5-HT has complex and somewhat variable effects upon chemosensory discharge in anaesthetized cats. We identified three separate components in the response to 5-HT and found that the 5-HT antagonists MDL 72222 and ketanserin selectively affected different parts of the response. Previous studies using respiration as an index have provided evidence for both inhibitory and excitatory effects of 5-HT on chemoreceptors in various species including cat (Page, 1952; Douglas & Toh, 1953; Ginzel & Kottegoda, 1954). 5-HT also induces complex neurogenic and circulatory effects which influence respiration independently of the chemoreceptors (Mott & Paintal, 1953; Comroe *et al.*, 1953), which makes the respiratory effect a poor index of chemoreceptor activity. We shall confine ourselves to a consideration of neural data, looking at the effects of antagonists on each of the components of the chemoreceptor response to 5-HT.

Chemoexcitation

Transient chemoexcitation occurred during the injection period in about half the recordings, and this effect has previously been described by Black *et al.* (1972), Nishi (1975), and Docherty & McQueen (1978). The increase was only occasionally dose-related and seemed subject to tachyphylaxis. The rapid onset suggests a direct or perhaps indirect (via release of endogenous substance(s)) action on the sensory nerve fibres (Eyzaguirre & Nishi, 1974), and makes it unlikely to be due to vascular effects of 5-HT. The possible influences on the chemoreceptors of increased sympathetic activity arising from the ganglion-stimulating action of 5-HT (Trendelenburg, 1958) were prevented by sectioning the gangliolomerular nerves. This does not exclude the possibility that 5-HT may release noradrenaline from the terminals of sympathetic nerves within the carotid body; such an action might be expected to reduce blood flow through the carotid body and could cause a *delayed* increase in discharge. The finding that transient excitation was not obtained in all recordings, in accord with Black *et al.* (1972), could be because fibres differ in their sensitivity: unmyelinated fibres may be more sensitive than myelinated, or the concentration of 5-HT at the receptor site following intracarotid injection may vary between experiments. Tachyphylaxis to 5-HT cannot explain the absence of excitation in response to the initial doses of 5-HT, although in recordings where excitation was occurring, repeated administration of 5-HT in high doses did tend to attenuate the response and may explain why there was no dose-response relationship in many experiments. Nishi

(1975), who evidently found transient chemoexcitation by 5-HT to be more common and induced by lower doses than was our experience, reported that various antagonists were ineffective in blocking the response. He was unable to characterize the receptor responsible for excitation, although he did exclude direct or indirect involvement of nicotinic and muscarinic ACh receptors. Docherty & McQueen (1978) found that the dopamine antagonist α-flupenthixol could reduce excitatory responses to 5-HT, but inconsistently. We have not studied putative 5-HT antagonists such as LSD or methysergide because of concern over their specificity, but instead used the newer 5-HT antagonists MDL 72222 and ketanserin. The latter had no appreciable effect on the transient chemoexcitation but MDL 72222 inhibited it. Thus in some, but not all recordings of chemoreceptor activity, 5-HT increases discharge transiently and this occurs via actions, directly or indirectly mediated, at a receptor, presumably within the carotid body and perhaps associated with sensory nerve endings, which is sensitive to the antagonist MDL 72222. Recent studies have shown that MDL 72222 blocks the excitatory action of 5-HT on the cell bodies of rabbit vagal primary afferents (Azami *et al.*, 1984), and this evidence supports the concept of 5-HT acting on neuronal or sensory receptors in the carotid body. The fact that MDL 72222 itself had some 5-HT-like actions could mean the drug is a partial agonist.

Chemodepression

5-HT caused a short-lasting period of chemodepression which commenced almost immediately upon completion of the injection and was dose-related in the majority of experiments. Again, the rapid onset of the effect makes it unlikely to be secondary to vascular changes caused by 5-HT. It was the most commonly encountered component of the response and occurred regardless of whether or not the initial excitation was present. Depression of chemoreceptor discharge has been demonstrated previously (Black *et al.*, 1972; Nishi, 1975; Docherty & McQueen, 1978). Earlier studies showed that very high doses of α-flupenthixol reduced the relative inhibition caused by 5-HT (Docherty & McQueen, 1978), but none of the putative 5-HT antagonists examined by Nishi (1975) had any effect. His suggestion that depression might be secondary to the initial excitation seems unlikely, for if this were the case, chemodepression should not occur in the absence of an initial depolarization, yet it did in our experiments. We found that ketanserin had a rather variable effect upon 5-HT-induced chemodepression, usually causing a slight reduction (although sometimes a potentiation) of the effect. In contrast, MDL 72222 caused a substantial dose-related antagonism of chemodepression, as shown by the increase in ID_{50} values, and higher doses ($100\ \mu g\ kg^{-1}$) could completely abolish the response, unmasking an excitatory component. Dopamine also causes chemodepression when injected in cats (Docherty & McQueen, 1978), and in view of the fact that high doses of α-flupenthixol can reduce responses to 5-HT as well as to dopamine, we examined the responses to dopamine and 5-HT before and after administering the selective dopamine D_2-receptor (Kebabian & Calne, 1979) antagonist, domperidone. Domperidone had no significant effect on any phase of the chemoreceptor response to 5-HT when given in doses which substantially reduced dopamine-induced chemodepression, and responses to dopamine were unaffected by either MDL 72222 or ketanserin. Thus, we can conclude that chemodepression evoked by 5-HT does not involve a dopamine D_2-receptor and is mainly mediated by mechanisms which are sensitive to MDL 72222. The results with ketanserin could mean that a small part of the depression is attributable to actions on $5\text{-}HT_2$-receptors, assuming the antagonist is selective and does not affect MDL 72222-sensitive sites in the doses studied. Whether the depression of discharge results from direct actions of 5-HT, or is secondary to the release of an inhibitory substance, cannot be determined from our study.

Delayed excitation

The final component of the response to injected 5-HT was a delayed (10–30 s) increase in discharge that lasted longer than any of the other components. However, it was very variable and tended to be concurrent with the fall in blood pressure caused by 5-HT. The antagonist MDL 72222 had no effect on blood pressure responses to 5-HT or on the delayed excitation whereas ketanserin inhibited both the hypotensive effect and the increase in discharge. The chemoexcitation caused by ketanserin alone may reflect some partial agonist activity of the drug. We cannot tell from the data whether the delayed chemoexcitation was due to the hypotension caused by 5-HT, or resulted from actions of the amine on $5\text{-}HT_2$-receptors in the carotid body, perhaps associated with the vasculature (Leysen *et al.*, 1981) or the nerves (e.g. sympathetic terminals – see earlier discussion). Further experiments are needed to resolve the matter.

Classification of 5-HT receptors

In the peripheral nervous system classification of 5-HT receptors is complicated (see Wallis, 1981) and, in addition, new antagonists such as MDL 72222

have yet to be fully characterized *in vivo*. Accordingly, we can only conclude that at least two 5-HT receptors appear to be responsible for changes in carotid chemosensory discharge evoked by injected 5-HT. It is not possible to say whether or not the transient excitation and depression are mediated through a common MDL 72222-sensitive mechanism, but overall our findings are consistent with reports showing that MDL 72222 is a selective antagonist of responses mediated through 5-HT receptors on peripheral nerves (Fozard, 1984) and that ketanserin appears to be an effective antagonist at vascular 5-HT_2-receptors (Leysen *et al.*, 1981).

Physiological stimulation

Although the combination of MDL 72222 and ketanserin antagonized the effects of exogenous 5-HT on chemosensory discharge, the response of the chemoreceptors to physiological stimulation by hypoxia was unaltered. Assuming the antagonists studied reach effective concentrations at sites within the carotid body where locally-released 5-HT acts, the implication is that endogenous 5-HT has no vital role in the mechanism of chemoreception. However, the possibility that 5-HT might exert subtle influences, perhaps as a modulator or co-transmitter, that were not detected in these experiments, cannot entirely be excluded. Neuronal co-storage of 5-HT with polypeptides, some of which are present in the cat carotid body (e.g. substance P; Cuello & McQueen, 1980), and may be neurotransmitters (see Hökfelt *et al.*, 1980), could mean that if 5-HT is involved in chemoreception it may function more as a modulator than as a 'primary' transmitter. The conditions of our experiments do not allow us to reach any conclusions on this possibility, nor on the question of whether 5-HT may be released within the carotid body by efferent nerves. However, the abundance of 5-HT in the carotid body does imply that it has some function in this organ and further studies seem warranted.

Local blood flow within the carotid body may be important in determining chemoreceptor discharge (Joels & Neil, 1963), so 5-HT could be more involved in regulating blood flow than in exerting a direct influence on the chemoreceptor cell-sensory nerve ending complex. Haemodynamic responses to 5-HT may be more subtle than simple changes in vascular tone. For example, 5-HT can increase vascular permeability, resulting in fluid-leakage into the perivascular spaces, and local haemoconcentration ('stasis') within the vessel (Majno & Palade, 1961; Majno *et al.*, 1961). It is doubtful whether such an action would be rapid enough, or sufficiently transient, to explain adequately any of the 5-HT effects we observed, but is indicative of the complex mechanisms that might mediate apparently simple effects of 5-HT. Histochemical studies in rats have confirmed the presence of 5-HT in carotid body type 1 cells, particularly those clustered around blood vessels (Grönblad *et al.*, 1983), so it is conceivable that 5-HT released from type 1 cells acts on 5-HT_2-receptors to alter vascular tone. Such changes in 5-HT output may have important consequences in certain pathophysiological states, such as hypertension (see Steele & Hinterberger, 1972).

In conclusion, 5-HT affects chemosensory discharge and at least two types of receptor appear to be involved in the responses evoked. Further studies with the new specific 5-HT agonists and antagonists, and utilizing neuropharmacological, ligand-binding and histochemical techniques, together with selective denervation of the carotid body, should establish where within this sensory organ these 5-HT receptors are located.

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EFFECTS OF A NEW 5-HYDROXYTRYPTAMINE (5-HT) ANTAGONIST, MDL 72222, ON RESPONSES OF CAT CAROTID CHEMORECEPTORS TO 5-HT AND HYPOXIA

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The cat carotid body contains 5-HT, and exogenous 5-HT has complex effects on carotid chemoreceptor activity. In order to characterise the receptors involved in the response to 5-HT we have used a new 5-HT antagonist, MDL 72222 (MDL); previous antagonists were not selective enough for receptor characterisation. 5-HT (1-50 µg i.c.) elicited three distinct effects on chemosensory discharge recorded from filaments of the carotid sinus nerve in anaesthetised cats. In all experiments chemodepression occurred, and this was preceded by a transient burst of activity in 56% of recordings. The third component involved a delayed slight increase in discharge, associated with hypotension. MDL (10-100 µg kg^{-1} i.c.) itself caused a brief period of chemodepression and delayed secondary excitation. Following MDL there was a dose-related antagonism of both the transient excitation and the chemodepression evoked by 5-HT, without any change in the delayed excitation or associated hypotension. The mean ID_{50} for 5-HT-induced chemodepression increased from 5.8 ± 1.9 µg (± s.e.m.; n=12) before, to 49.4 ± 33.6 (n=7; $P < 0.05$) after 10 µg kg^{-1} antagonist, and 638 ± 408 (n=5; $P < 0.05$) after 100 µg kg^{-1}. The increase in chemosensory discharge caused by ventilating the animal with 10% O_2 instead of air was not significantly affected by MDL (10-100 µg kg^{-1} i.c.).

Carotid chemoreceptors are affected by 5-HT, and the greater part of the response involves MDL-sensitive (neuronal) receptors. However, the fact that the antagonist has no appreciable effect on the responsiveness of the chemoreceptors to a physiological stimulus such as hypoxia suggests it is unlikely that endogenous 5-HT, acting via MDL-sensitive receptors has an important role in chemoreception, although it might be a modulator.

MDL was kindly donated by Merrell, Strasbourg.

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EFFECTS OF SUBSTANCE P ON RESPONSES OF CAT CAROTID BODY CHEMORECEPTORS TO DOPAMINE, NORADRENALINE AND 5-HYDROXYTRYPTAMINE

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The cat carotid body contains substance P (SP)-like material (Cuello & McQueen, 1980) as well as the putative transmitters dopamine (DA), noradrenaline (NA), and 5-hydroxytryptamine (5-HT). Preliminary investigations showed that SP can modify spontaneous discharge and some drug-induced responses of the carotid chemoreceptors (McQueen, 1980), and we have now studied the influence of SP on responses of cat carotid chemoreceptors to DA, NA and 5-HT. Experiments were performed on pentobarbitone-anaesthetized cats (42 mg kg^{-1} i.p., supplemented as necessary), artificially ventilated and paralysed with gallamine (3 mg kg^{-1} i.v.). Chemosensory discharge was recorded from the peripheral end of a cut sinus nerve, and drugs were injected or infused into the common carotid artery as previously described (McQueen, 1980). Infusions of SP (10 µg min^{-1}) or drug vehicle (Locke solution) were made at a rate of 0.1 ml min^{-1} for 2 min and injections of the amines made 90 s after starting the infusion. This protocol was chosen to minimise the tachyphylaxis caused by SP.

Within 15 s of onset of SP infusion there was a small but significant decrease ($P < 0.05$) in spontaneous discharge from the pre-infusion control frequency; Locke solution had no marked effect on discharge.

NA (0.1 - 50 µg i.c.) caused chemodepression followed by chemoexcitation; chemodepression evoked by the higher, but not the lower doses of NA was potentiated during SP infusion, whereas the delayed chemoexcitation was reduced. 5-HT (1 - 25 µg i.c.) caused dose-related chemodepression that was potentiated during SP infusion. Secondary excitation following chemodepression was smaller in magnitude than that associated with NA, but, in contrast, was potentiated during SP infusion. DA (0.1 - 10 µg i.c.) induced dose-related chemodepression which was greater than that evoked by NA or 5-HT, but the effect was largely unaltered during SP infusion.

Our results indicate that SP-monoamine interactions are rather complex, and there is evidence for a differential effect of the peptide on the responsiveness of carotid chemoreceptors to the amines studied. Thus, SP potentiated NA and 5-HT-induced chemodepression, but had no effect on the depressant action of DA. Delayed chemoexcitation caused by NA was reduced during SP infusion, but that evoked by 5-HT was potentiated. It remains to be established whether SP modifies chemoreceptor activity by acting directly on some element of the chemosensory complex within the carotid body rather than indirectly by a non-specific action (e.g. vascular effects which in turn influence chemoreceptor discharge). Further studies are needed to determine whether the peptide-amine interactions described have any physiological significance, and also to characterize the type(s) of SP receptor involved in modifying chemoreceptor activity.

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Effects of selective β-adrenergic antagonists on the response of carotid chemoreceptors to hypoxia in anaesthetized cats

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Folgering, Ponte & Sadig (1982) reported that propranolol reduced or abolished hypoxia-induced chemo-excitation in cats, but Gonsalves, Smith, Nolan & Dutton (1983) found no clear attenuation of this response by propranolol. We have studied the effects of selective β-adrenoceptor antagonists on steady-state and dynamic responses of carotid chemoreceptors to hypoxia. Chemosensory discharge was recorded in pentobarbitone-anaesthetized cats (42 mg kg^{-1} I.P., see McQueen, 1977). Discharge during normoxia, plateau (maximum) discharge during 4 min ventilation with 10% O_2, and the rate of increase of discharge in response to hypoxia were measured before and after injection of an antagonist (Table 1).

Table 1

	$n = 3$		$n = 5$	
	Control	Propranolol	Control	Betaxolol
Discharge (air) (c.p.s. ± S.E. of mean)	6·7 ± 2·9	3·8 ± 0·8	6·9 ± 1·6	7·3 ± 2·2
Discharge (10% O_2) (c.p.s. ± S.E. of mean)	26·8 ± 12·2	12·1 ± 2·9	29·8 ± 9·2	29·5 ± 9·5
Rate of Increase (% max. s^{-1} ± S.E. of mean)	0·62 ± 0·12	0·36 ± 0·09	0·57 ± 0·06	0·57 ± 0·06

Single injections of propranolol (1·6–5·8 mg kg^{-1} I.V.) tended to reduce normoxic and hypoxic discharge, but responses after the drug were not statistically different from the controls ($P > 0·05$, paired t test). Since propranolol has membrane-stabilizing properties we studied the effects of betaxolol, a selective β_1 antagonist which has only weak local anaesthetic properties (Boudot, Cavero, Fénard, Lefèvre-Borg, Manoury & Roach, 1979). A dose of 1 mg kg^{-1} blocked both the chemoreceptor excitation and the increased arterial pulse pressure elicited by intracarotid injection of noradrenaline (1–50 μg) but did not significantly alter any of the parameters measured. During ventilation with 10% O_2 mean P_{a,O_2} values were 4·99 ± 0·7 kPa before and 5·25 ± 0·4 kPa after betaxolol: corresponding P_{a,CO_2} values were 3·90 ± 0·1 kPa and 3·52 ± 0·2 kPa ($n = 4$; $P > 0·05$, paired t tests). ICI 118551, a selective β_2 antagonist, caused no significant reduction in the chemoreceptor response to hypoxia.

Assuming the antagonists in the doses used are capable of blocking the actions of endogenous catecholamines, the present results do not support the direct involvement of a β-receptor-mediated mechanism in the chemo-excitation evoked by hypoxia.

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EFFECTS OF SELECTIVE 5-HYDROXYTRYPTAMINE AGONISTS ON CAROTID BODY CHEMORECEPTOR DISCHARGE IN ANAESTHETIZED CATS

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Intracarotid (i.c.) injection of 5-hydroxytryptamine (5-HT) in cats has the following effects on chemosensory discharge: dose-related chemodepression, selectively blocked by the 'neuronal' 5-HT antagonist MDL 72222 (MDL); delayed chemoexcitation, selectively blocked by the 5-HT_2 antagonist ketanserin, and intense, but transient chemoexcitation preceding 5-HT-evoked chemodepression, which is also selectively blocked by MDL (Kirby & McQueen, 1984). We have further studied the 5-HT receptors that might be responsible for these effects using the following selective agonists: 2-Methyl,5-hydroxytryptamine (2Me5-HT), active at MDL-sensitive sites (see Humphrey, 1984); 5-methoxytryptamine (5-MeT), which is inactive at MDL-sensitive sites (Fozard, 1984); 8-OH-DPAT, a 5-HT_{1A} agonist (Middlemiss & Fozard, 1983), and RU 24969, a selective 5-HT_{1B} agonist (Cortés et al, 1984).

Chemoreceptor discharge was recorded in pentobarbitone-anaesthetized cats, artificially ventilated with air and paralysed with gallamine (for details see McQueen, 1977), and responses evoked by i.c. injections of the 5-HT agonists were compared.

During experiments in which the typical effects of injection of 5-HT (1-25 µg) were obtained, 2Me5-HT (1-50 µg; n=4) caused chemodepression equal to, or greater than that seen with 5-HT; marked transient chemoexcitation was elicited by 5-HT in all four experiments, whereas 2Me5-HT had only weak effects. 2Me5-HT caused variable secondary chemoexcitation, and hypotension similar to that induced by 5-HT. In contrast, 5-MeT (1-100 µg; n=3) caused only slight changes in chemoreceptor discharge, although its vascular effects were similar to those evoked by 5-HT.

RU 24969 (0.5-50 µg; n=2) caused marked dose-dependent prolonged chemoexcitation, preceded in one experiment by slight chemodepression - less than that seen with 5-HT. There was no initial transient chemoexcitation, nor any obvious effect on blood pressure. 8-OH-DPAT (0.5-50 µg; n=2) caused prolonged dose-related hypotension; there was neither initial transient nor delayed chemoexcitation, but chemodepression, greater than that caused by 5-HT occurred, and persisted after 5-HT-evoked chemodepression had been blocked by MDL (100 µg kg^{-1}) in one of the experiments.

The rank order of potency of these agonists for the three phases of the '5-HT response' appears to be: transient excitation 5-HT >> 2Me5-HT; chemodepression 8-OH-DPAT > 2Me5-HT and 5-HT >> RU24969 and 5MeT; secondary excitation RU 24969 > 5-HT > 2Me5-HT >> 8-OH-DPAT and 5-MeT. These results provide further evidence that 'neuronal' 5-HT receptors are associated with the carotid chemoreceptors, and also emphasise the complexity of the 5-HT effects on chemosensory discharge, the significance of which remains to be determined.

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ICI 154,129 antagonizes [met]enkephalin-induced depression of cat chemosensory discharge

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[Met]enkephalin appears to be present in the cat carotid body (Wharton *et al.* 1980). Intracarotid injection of the peptide depresses chemosensory discharge in cats, and this effect is antagonized by naloxone (McQueen & Ribeiro, 1980). In the present study we have used the selective δ opioid receptor antagonist ICI 154,129 (Shaw *et al.* 1982) to help characterize the type of opioid receptor involved in the enkephalin-induced depression of chemosensory discharge.

Experiments were performed on cats anaesthetized with pentobarbitone (42 mg kg^{-1} I.P.), artificially ventilated with air, and paralysed with gallamine (3 mg kg^{-1} I.V.). Chemosensory discharge was recorded from the peripheral end of a sectioned carotid sinus nerve using techniques previously described (see McQueen & Ribeiro, 1980). Drugs were injected into the common carotid artery close to the carotid body from which chemoreceptor activity was recorded and the averaged discharge frequency during the 30 s post-injection period was expressed as a percentage change from the pre-injection discharge. The ID_{50} (dose causing a 50% reduction in discharge over the 30 s period) was calculated for [Met]enkephalin from dose–response lines using data obtained before and after administering ICI 154,129 (0·1–10 mg kg^{-1} I.C.).

The mean ID_{50} for [Met]enkephalin-induced chemodepression was $0{\cdot}89 \pm 0{\cdot}16$ (S.E. of mean, $N = 9$) nmol before the antagonist. After ICI 154,129 in doses of 0·1, 1 and 10 mg kg^{-1} I.C. the mean ID_{50} values were $1{\cdot}01 \pm 0{\cdot}38$ ($N = 3$), $4{\cdot}32 \pm 1{\cdot}27$ ($N = 4$) and $72{\cdot}5 \pm 26{\cdot}1$ ($N = 6$) nmol respectively. ICI 154,129 itself had only slight effects on discharge, tending to increase discharge at the low dose and decrease it after the high dose. The intermediate dose of 1 mg kg^{-1} had no significant effect; none of the doses had any appreciable effect on arterial blood pressure.

The finding that ICI 154,129 causes dose-related antagonism of [Met]enkephalin-induced chemosensory depression is consistent with actions of the opioid at δ type receptors in the carotid body.

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